Наборы для биохимического анализа, реагентов для иммуноферментного анализа, для анализа коллоидного золота, адъювант

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Biochemistry & Immunology

Biochemical Assay Kit

ELISA Kits

Human | Mouse | Rat | Rabbit | General | ELISA Stop Solution

Colloidal Gold Assay Kit

Colloidal Gold Assay Kit

Antibody Preparation

Adjuvant

Products>Biochemistry & Immunology>Biochemical Assay Kit

Cat.No.	Product Name	Spec.
G4300-48T	Malondialdehyde (MDA) Assay Kit(for Cell samples)	48T
G4300-96T	Malondialdehyde (MDA) Assay Kit(for Cell samples)	96 T
G4301-48T	Total Iron Ion Assay Kit	48 T
G4301-96T	Total Iron Ion Assay Kit	96 T
G4302-48T	Malondialdehyde (MDA) Assay Kit (for Tissue and Blood)	48 T
G4302-96T	Malondialdehyde (MDA) Assay Kit (for Tissue and Blood)	96 T
G4303-48T	Total Glutathione (T-GSH) Assay Kit	48 T
G4303-96T	Total Glutathione (T-GSH) Assay Kit	96 T
G4304-48T	Total Glutathione/Oxidized Glutathione Assay Kit	48 T
G4304-96T	Total Glutathione/Oxidized Glutathione Assay Kit	96 T
G4305-48T	Reduced Glutathione (GSH) Assay Kit	48 T
G4305-96T	Reduced Glutathione (GSH) Assay Kit	96 T
G4306-48T	Total Superoxide Dismutase (T-SOD) Activity Assay Kit	48 T
G4306-96T	Total Superoxide Dismutase (T-SOD) Activity Assay Kit	96 T
G4307-48T	Catalase (CAT) Activity Assay Kit	48 T
G4307-96T	Catalase (CAT) Activity Assay Kit	96 T
G4309-48T	ATP Assay Kit (ChemiLuminescence)	48 T

Products>Biochemistry & Immunology>ELISA Kits

Products>Biochemistry & Immunology>ELISA Kits>Human

Cat.No.	Product Name	Spec.
GE0001-48T	HIV-1 Gag p24 ELISA Kit	48 T
GE0001-96T	HIV-1 Gag p24 ELISA Kit	96 T
GEH0001-48T	Human IL-6 ELISA Kit	48 T
GEH0001-96T	Human IL-6 ELISA Kit	96 T
GEH0002-48T	Human IL-1 beta ELISA Kit	48 T
GEH0002-96T	Human IL-1 beta ELISA Kit	96 T

GEH0003-48T	Human IL-10 ELISA Kit	48 T
GEH0003-96T	Human IL-10 ELISA Kit	96 T
GEH0004-48T	Human TNF-alpha ELISA Kit	48 T
GEH0004-96T	Human TNF-alpha ELISA Kit	96 T
GEH0005-48T	Human IL-8 ELISA Kit	48 T
GEH0005-96T	Human IL-8 ELISA Kit	96 T
GEH0006-48T	Human IFN-gamma ELISA Kit	48 T
GEH0006-96T	Human IFN-gamma ELISA Kit	96 T
GEH0008-48T	Human IL-4 ELISA Kit	48 T
GEH0008-96T	Human IL-4 ELISA Kit	96 T
GEH0009-48T	Human IL-12 p70 ELISA Kit	48 T
GEH0009-96T	Human IL-12 p70 ELISA Kit	96 T
GEH0010-48T	Human IL-18 ELISA Kit	48 T
GEH0010-96T	Human IL-18 ELISA Kit	96 T
GEH0016-48T	Human IL-17A ELISA Kit	48 T
GEH0016-96T	Human IL-17A ELISA Kit	96 T
GEH0038-48T	Human IL-2 ELISA Kit	48 T
GEH0038-96T	Human IL-2 ELISA Kit	96 T

Cat.No.	Product Name	Spec.
GEM0001-48T	Mouse IL-6 ELISA Kit	48 T
GEM0001-96T	Mouse IL-6 ELISA Kit	96 T
GEM0002-48T	Mouse IL-1 beta ELISA Kit	48 T
GEM0002-96T	Mouse IL-1 beta ELISA Kit	96 T
GEM0003-48T	Mouse IL-10 ELISA Kit	48 T
GEM0003-96T	Mouse IL-10 ELISA Kit	96 T
GEM0004-48T	Mouse TNF-alpha ELISA Kit	48 T
GEM0004-96T	Mouse TNF-alpha ELISA Kit	96 T
GEM0006-48T	Mouse IFN-gamma ELISA Kit	48 T
GEM0006-96T	Mouse IFN-gamma ELISA Kit	96 T
GEM0007-48T	Mouse C-Reactive Protein (CRP) ELISA Kit	48 T
GEM0007-96T	Mouse C-Reactive Protein (CRP) ELISA Kit	96 T
GEM0008-48T	Mouse IL-4 ELISA Kit	48 T
GEM0008-96T	Mouse IL-4 ELISA Kit	96 T
GEM0009-48T	Mouse IL-12 p70 ELISA Kit	48 T
GEM0009-96T	Mouse IL-12 p70 ELISA Kit	96 T
GEM0010-48T	Mouse IL-18 ELISA Kit	48 T
GEM0010-96T	Mouse IL-18 ELISA Kit	96 T

GEM0013-48T	Mouse IgG ELISA Kit	48 T	
GEM0013-96T	Mouse IgG ELISA Kit	96 T	
GEM0016-48T	Mouse IL-17A ELISA Kit	48 T	
GEM0016-96T	Mouse IL-17A ELISA Kit	96 T	
GEM0017-48T	Mouse CCL2/MCP-1 ELISA Kit	48 T	
GEM0017-96T	Mouse CCL2/MCP-1 ELISA Kit	96 T	
GEM0018-48T	Mouse IFN-beta ELISA Kit	48 T	
GEM0018-96T	Mouse IFN-beta ELISA Kit	96 T	
GEM0021-48T	Mouse MMP-9 ELISA Kit	48 T	
GEM0021-96T	Mouse MMP-9 ELISA Kit	96 T	
GEM0022-48T	Mouse VEGF ELISA Kit	48 T	
GEM0022-96T	Mouse VEGF ELISA Kit	96 T	
GEM0024-48T	Mouse CXCL1 ELISA Kit	48 T	

Products>Biochemistry & Immunology>ELISA Kits>Rat

Cat.No.	Product Name	Spec.	
GER0001-48T	Rat IL-6 ELISA Kit	48 T	
GER0001-96T	Rat IL-6 ELISA Kit	96 T	
GER0002-48T	Rat IL-1 beta ELISA Kit	48 T	
GER0002-96T	Rat IL-1 beta ELISA Kit	96 T	
GER0003-48T	Rat IL-10 ELISA Kit	48 T	
GER0003-96T	Rat IL-10 ELISA Kit	96 T	
GER0004-48T	Rat TNF-alpha ELISA Kit	48 T	
GER0004-96T	Rat TNF-alpha ELISA Kit	96 T	
GER0006-48T	Rat IFN-gamma ELISA Kit	48 T	
GER0006-96T	Rat IFN-gamma ELISA Kit	96 T	
GER0007-48T	Rat C-Reactive Protein (CRP) ELISA Kit	48 T	
GER0007-96T	Rat C-Reactive Protein (CRP) ELISA Kit	96 T	
GER0013-96T	Rat IgG ELISA Kit	96 T	

Products>Biochemistry & Immunology>ELISA Kits>General

Cat.No.	Product Name	Spec.	
GE0002-48T	Cortisol ELISA Kit (Common Species)	48 T	
GE0002-96T	Cortisol ELISA Kit (Common Species)	96 T	

Products>Biochemistry & Immunology>ELISA Kits>ELISA Stop Solution

Cat.No.	Product Name	Spec.	
G0027-100ML	ELISA Stop Solution	100 mL	

Products>Biochemistry & Immunology>Colloidal Gold Assay Kit

Cat.No.	Product Name	Spec.
G1804-10T	Rapid Lentivirus Titration Cassette (Colloidal Gold)	10 T
G1906-10T	His-tag Rapid Assay (Colloidal Gold)	10 T
G1907-10T	Flag-tag Rapid Assay (Colloidal Gold)	10 T
G1908-10T	HA-tag Rapid Assay (Colloidal Gold)	10 T
G1909-10T	S-tag Rapid Assay (Colloidal Gold)	10 T

Cat.No.	Product Name	Spec.
G1910-10T	V5-tag Quick Detection Card (Colloidal Gold Method)	10 T
G1911-10T	Sumo-tag Quick Detection Card (Colloidal Gold Method)	10 T

Cat.No.	Product Name	Spec.
G4704-5ML	Ultrafast Response Aqueous Adjuvant	5 mL

Servicebio® Mouse IgG ELISA Kit

Cat. #：GEM0013

Product Information

Product Name	Cat. No.	Spec.
Mouse IgG ELISA Kit	GEM0013-48T	48T
	GEM0013-96T	96T

Product Description

ImmunoglobulinG (IgG) is the most important form of immunoglobulin in serum, accounting for about 75% of the total content of immunoglobuling in serum, and is the most important antibody component in serum and extracellular fluid, mainly involved in the identification and elimination of pathogens and toxic substances, and has antiviral, antibacterial and immunomodulatory functions.IgG is a four-stranded monomer, consisting of two light and two heavy chains.Structurally, it can be divided into variable region and constant region, wherein variable region has antigen recognition site.IgG is also the only antibody that crosses the placenta during pregnancy to protect the fetus and plays an important role in fighting infection in the newborn.Mouse IgG ELISA Kit can quantitatively detect IgG in mouse serum, plasma, cell culture supernatant or other related liquids in vitro through double antibody sandwich ELISA technique, and can detect both natural and recombinant mouse IgG.

删除[a]: tissue homogenate, cell lysate,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GEH0014-48T	GEH0014-96T
GEM0013-1	Microplate	48T	96T
GEM0013-2	Standard	1 vial	2 vials
G0030	Enzyme-labeled Antibody	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1pc	1pc

删除[丽]: Precoated Enzyme Plates

删除[丽]: each

删除[丽]: each

删除[丽]: 1pc

删除[丽]:

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm(Refer to the instruction manual supplied with the instrument to pre-warm).

2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate (25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Preparation of standard product: Add diluent A to the standard product according to the volume marked on the label, and gently swirl to ensure full mixing.The standard product concentration after redissolution is 100ng/mL, that is, the concentrated mouse IgG standard product. Let stand for 10 minutes after remelting and mix well before diluting.
   a) Preparation of standard curves for serum/plasma:
      After fully mixing the redissolved standard product, 500μL concentrated mouse IgG standard product was taken and added with 500μL diluent A as the highest concentration S7 (50ng/mL) on the standard curve.Six 1.5mL centrifuge tubes (S1-S6) were arranged successively, and 500μL diluent A was added to each tube.Draw 500μLS7 (50ng/mL) standard into the first centrifuge tube S6 and gently blow and mix.Draw 500μL from S6 into the second centrifuge tube S5 and gently blow and mix.And so on to double dilution of standard products.S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
      After fully mixing the redissolved standard product, 500μL concentrated mouse IgG standard product was taken and added into 500μL cell medium as the highest concentration S7 (50ng/mL) of the standard curve.Six 1.5mL centrifuge tubes (S1-S6) were sequentially arranged and 500μL cell medium was added to each.Draw 500μLS7 (50ng/mL) standard into the first centrifuge tube

删除[a]: **Tissue homogenates:** The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

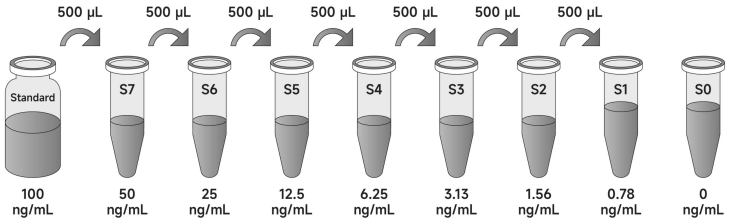
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 μL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 μL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

S6 and gently blow and mix.Draw 500μL from S6 into the second centrifuge tube S5 and gently blow and mix.And so on to double dilution of standard products.S0 is the cell medium.



4. 1× enzymic antibody: The enzymic antibody is briefly centrifuged and diluted to the working concentration by 1:100 times with diluent B.1× enzyme-labeled antibody working liquid was mixed and prepared before clinical use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Standard holes, sample holes to be measured and blank holes are set up respectively.Dilute the standard product and sample with diluent A, set standard holes 7 holes (S1-S7), add 100μL standard product of different concentration to each hole successively, add 100μL diluent A to each hole, add 100μL sample to each hole, seal the plate membrane, and oscillate at 100-300rpm (ensure that the solution does not spill out of each hole and can be fully mixed).Incubate at room temperature for 2 hours;

   Note: Please refer to relevant literature to determine the approximate concentration of the protein to be detected in the sample. If the concentration is greater than or less than the maximum or minimum standard concentration of this kit, please carry out appropriate dilution or concentration before testing.
2. **Wash plate:** Automatic washing plate or manual washing plate, each hole of the washing liquid is 300μL, injection and suction interval of 15-30 seconds.Wash the board five times.After the last plate washing is completed, the enzyme label plate is upside-down on the absorbent paper and patted dry properly, and the liquid in the hole is discarded;
3. **Add the enzyme-labeled antibody:** Dilute the enzyme-labeled antibody to the working concentration with diluant B, add the enzyme-labeled antibody working solution (prepared before use) 100μL in each well, replace the sealing plate with a new sealing plate, shake at 100-300rpm (ensure that the solution does not spill in each well and can be fully mixed), and incubate at room temperature for 30 minutes;
4. **Plate washing:** Repeat step 2.

5. **Add TMB Substrate:** Add TMB substrate solution 90μL to each hole, replace the new sealing plate film, and color rendering at room temperature (the reaction time should be controlled within 10-30 minutes, not more than 30 minutes.When the first 3-4 holes of the standard product have obvious gradient blue, the gradient of the last 3-4 holes is not obvious, it can be terminated);
6. **Add Stop Solution:** Add termination solution 50μL to each well to terminate the reaction, and the blue immediately turns to yellow.The addition sequence of the termination solution should be as close as possible to the addition sequence of the substrate solution.If the color is uneven, please gently shake the label plate to make the solution evenly mixed;
7. **Reading:** .After ensuring that there are no water droplets at the bottom of the enzyme label plate and no bubbles in the hole, the detection wavelength of 450nm is used to read the value within 10 minutes.It is recommended to use dual wavelengths, namely the detection wavelength of 450nm, the reference wavelength, or the correction wavelength of 630nm to read the value at the same time. Using only 450nm will reduce the accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.

b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

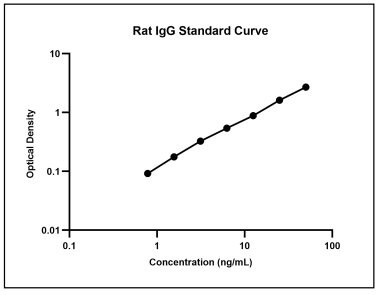
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

ng/mL	OD		Average	Corrected
50	2.725	2.743	2.734	2.706
25	1.598	1.690	1.644	1.616
12.5	0.915	0.909	0.912	0.884
6.25	0.566	0.574	0.570	0.542
3.13	0.354	0.356	0.355	0.327
1.56	0.204	0.203	0.204	0.176
0.78	0.124	0.116	0.120	0.092
0	0.028	0.027	0.028	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add sample (standard or sample) 100μL/ well, oscillate at room temperature for 2 hours;
3. After washing for 5 times, pat dry, add enzyme-labeled antibody working solution 100μL/ well, and shake at room temperature for 30 minutes;
4. Wash for 5 times and pat dry, add TMB substrate 90μL/ well, incubate at room temperature for 10-30 minutes away from light;
5. Add termination solution 50μL/ well;
6. The OD value is detected at 450 nm wavelength within 10 minutes, with a reference wavelength of 630 nm.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										

删除[a]: **Sensitivity**

The lowest detectable concentration of mouse IgG was 0.075ng/mL(the mean of 3 independent experiments).This value is the average OD value measured by 20 blank holes plus the concentration value corresponding to twice SD.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<9%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<15%.

Spike recovery

A certain amount of mouse IgG (labeled sample) was added to 5 samples of fixed mouse serum, repeated determination was made and the mean value was calculated. The serum without increasing mouse IgG was used as the background, and the recovery rate was calculated (the ratio of measured value to theoretical value).Recovery rates range from 90% to 99%, with an average recovery rate of 93%.

Linearity of dilution

A certain amount of mouse IgG (labeled sample) was added to 5 samples of fixed mouse serum, and a series of dilution was performed within the kinetic range of the standard curve to evaluate the linearity of the detection.The linear range is the ratio of the measured and theoretical IgG content of mouse in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
98
89-108

1:4
103
94-109

1:8
96
90-109

1:16
90
85-105

Sample values

Serum samples from 30 healthy mice were analyzed with

C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

删除[a]: V1

删除[a]: 202305

Servicebio® Mouse IL-17A ELISA Kit

Cat. #: GEM0016

Product Information

Product Name	Cat. No.	Spec.
Mouse IL-17A ELISA Kit	GEM0016-48T	48T
	GEM0016-96T	96T

Product Description/Introduction

Interleukin 17 (IL-17) is secreted by CD4+ T cells and is able to induce epithelial cells, endothelial cells, and fibroblasts to synthesise and secrete IL-6, IL-8, G-CSF, and PGE2, and to promote the expression of ICAM-1. The IL-17 family consists of six structurally related cytokines, IL-17A, IL-17B, IL -17C, IL-17D, IL-17E (IL-25), and IL-17F. Among the six members of the IL-17 family, IL-17A is the prototype of the IL-17 family, IL-17F has the highest homology and the coding gene is localised in the same region of the chromosome, 6p12, while the others are less homologous to IL-17A and are localised on different chromosomes. However, these cytokines are more conserved between human and murine species. Members of the IL-17 family function as homodimers or heterodimers. IL-17A is an important pro-inflammatory factor. Mouse IL-17A ELISA Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitative detection of IL-17A in mouse serum, plasma, cell culture supernatant or other related fluids, which can simultaneously detect natural and recombinant mouse IL-17A.

删除[丽]: tissue homogenate, cell lysate,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product components

Component Number	Component	GEM0016-48T	GEM0016-96T
GEM0016-1	Microplate	48T	96T
GEM0016-2	Standard	1 vial	2 vials
GEM0016-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample collection and storage instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated mouse IL-17A Standard is obtained by redissolving the standard to a concentration of 2000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.

a) Preparation of standard curves for serum/plasma:
Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated mouse IL-17A standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

b) Preparation of standard curve of cell culture supernatant sample:
Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated mouse IL-17A standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.

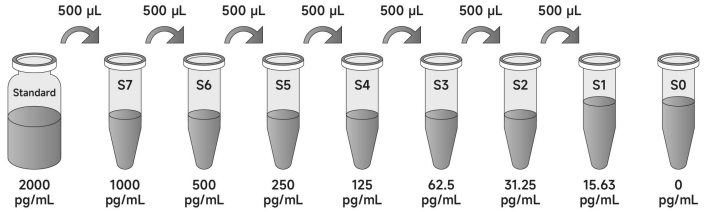
删除[丽]: **Tissue homogenates:** The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20℃ to avoid repeated freez-thaw cycles.

删除[丽]: /tissue homogenate/cell lysate



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol/Procedures

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 µL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.

7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation

coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

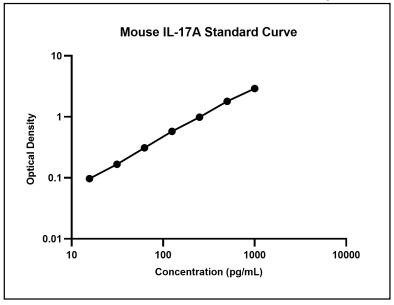
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
1000	2.916	2.938	2.927	2.904
500	1.814	1.823	1.819	1.796
250	1.069	0.949	1.009	0.986
125	0.613	0.589	0.601	0.578
62.5	0.338	0.327	0.333	0.310
31.25	0.195	0.182	0.189	0.166
15.63	0.114	0.126	0.120	0.097
0	0.021	0.025	0.023	



Test protocol summary

1. Prepare standards, reagents and samples according to instructions before the experiment.

2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.

3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.

4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.

5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.

6. Add 50 µL of stop solution to all wells.

7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[丽]: **Sensitivity**

The lower limit of detection of mouse IL-17A is 6.48 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV (%) =(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<8%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<10%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of mouse IL-17A into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 85% to 115%, with a mean recovery of 102%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of mouse IL-17A into serum. The linearity range is the ratio of the assay value to the expected value of mouse IL-17A in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
106
85-116

1:4
104
88-118

1:8
101
92-112

1:16
107
95-114

Sample values

The kit was applied to 30 normal mouse serum samples

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

For Research Use Only. Not for use in diagnostic procedures!

Version: V1.0-202306

Servicebio® Mouse CCL2/MCP-1 ELISA Kit

Cat. #：GEM0017

Product Information

Product Name	Cat. No.	Spec.
Mouse CCL2/MCP-1 ELISA Kit	GEM0017-48T	48T
	GEM0017-96T	96T

Product Description

Active Monocyte Chemotactic Protein 1 (MCP-1), also known as CCL2, belongs to the CC chemokine family and plays an important role in initiating and maintaining inflammatory responses. Its molecular structure includes a 23-amino acid signal peptide and a 76-amino acid mature peptide, secreted by various cells including epithelial cells, endothelial cells, smooth muscle cells, fibroblasts, and monocytes. MCP-1 recruits monocytes, memory T cells, and dendritic cells to sites of tissue damage or inflammation caused by infection, and participates in the pathogenesis of diseases characterized by monocyte infiltration, such as psoriasis, rheumatoid arthritis, and atherosclerosis. It also participates in neuroinflammatory processes in various diseases in the central nervous system, manifested as neuronal degeneration. The Mouse CCL2/MCP-1 ELISA Kit utilizes dual antibody sandwich enzyme-linked immunosorbent assay technology to quantitatively detect CCL2/MCP-1 in mouse serum, plasma, cell culture supernatants, or other related fluids, and can simultaneously detect both natural and recombinant mouse CCL2/MCP-1.

删除[a]: tissue homogenates, cell lysates,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GEM0017-48T	GEM0017-96T
GEM0017-1	Microplate	48T	96T
GEM0017-2	Standard	1 vial	2 vials
GEM0017-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

删除[丽]: each

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Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm(Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Mouse CCL2 Standard is obtained by redissolving the standard to a concentration of 2000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
      Mix the reconstituted standards well, add 500 µL of Diluent A to 500 µL of concentrated Mouse CCL2 standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Diluent A. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
      Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse CCL2 standard as the highest concentration S7 (1000 pg/mL) for the

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

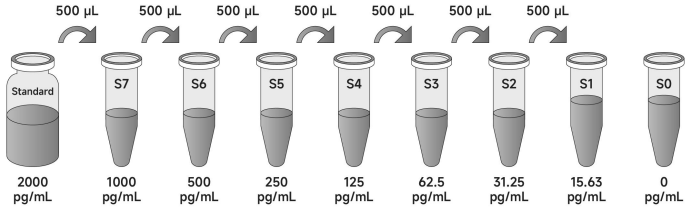
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 μL each of culture supernatant. Pipette 500 μL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 μL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 μL of standards of different concentrations to each well, add 100 μL of Diluent A to the blank wells, and 100 μL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 μL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Detection Antibody Addition:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 μL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.

5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

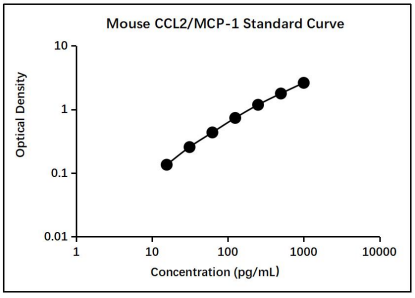
Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
1000	2.618	2.66	2.639	2.612
500	1.813	1.791	1.802	1.775
250	1.281	1.133	1.207	1.180
125	0.771	0.75	0.761	0.734
62.5	0.467	0.455	0.461	0.434
31.25	0.276	0.289	0.283	0.256
15.63	0.17	0.154	0.162	0.135
0	0.035	0.019	0.027	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										

删除[a]: **Sensitivity**

The lower limit of detection of mouse CCL2 is 1.96 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<6%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<9%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of mouse CCL2 into serum, repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 89% to 115%, with a mean recovery of 104%.

Linearity of dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of mouse CCL2 into serum. The linearity range is the ratio of the assay value to the expected value of mouse CCL2 in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
91
85-104

1:4
112
97-117

1:8
103
93-107

1:16
98
91-103

Sample values

The kit was applied to 30 normal mouse serum/plasma

B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

For Research Use Only!

Version: V2.0-202312

删除[a]: **V1**

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Tel: 027-5111-3188 Website: www.servicebio.com

Servicebio® Mouse CCL2/MCP-1 ELISA Kit

Cat. #：GEM0017

Product Information

Product Name	Cat. No.	Spec.
Mouse CCL2/MCP-1 ELISA Kit	GEM0017-48T	48T
	GEM0017-96T	96T

Product Description

Active Monocyte Chemotactic Protein 1 (MCP-1), also known as CCL2, belongs to the CC chemokine family and plays an important role in initiating and maintaining inflammatory responses. Its molecular structure includes a 23-amino acid signal peptide and a 76-amino acid mature peptide, secreted by various cells including epithelial cells, endothelial cells, smooth muscle cells, fibroblasts, and monocytes. MCP-1 recruits monocytes, memory T cells, and dendritic cells to sites of tissue damage or inflammation caused by infection, and participates in the pathogenesis of diseases characterized by monocyte infiltration, such as psoriasis, rheumatoid arthritis, and atherosclerosis. It also participates in neuroinflammatory processes in various diseases in the central nervous system, manifested as neuronal degeneration. The Mouse CCL2/MCP-1 ELISA Kit utilizes dual antibody sandwich enzyme-linked immunosorbent assay technology to quantitatively detect CCL2/MCP-1 in mouse serum, plasma, cell culture supernatants, or other related fluids, and can simultaneously detect both natural and recombinant mouse CCL2/MCP-1.

删除[a]: tissue homogenates, cell lysates,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GEM0017-48T	GEM0017-96T
GEM0017-1	Microplate	48T	96T
GEM0017-2	Standard	1 vial	2 vials
GEM0017-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

删除[丽]: each

删除[丽]: each

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm(Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Mouse CCL2 Standard is obtained by redissolving the standard to a concentration of 2000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
      Mix the reconstituted standards well, add 500 µL of Diluent A to 500 µL of concentrated Mouse CCL2 standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Diluent A. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
      Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse CCL2 standard as the highest concentration S7 (1000 pg/mL) for the

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

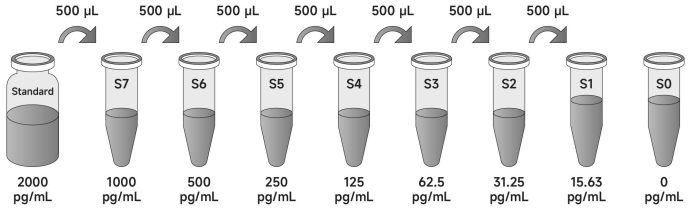
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 μL each of culture supernatant. Pipette 500 μL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 μL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 μL of standards of different concentrations to each well, add 100 μL of Diluent A to the blank wells, and 100 μL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 μL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Detection Antibody Addition:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 μL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.

5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

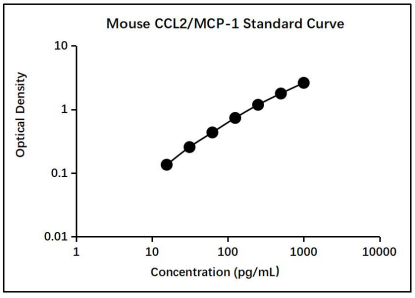
Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
1000	2.618	2.66	2.639	2.612
500	1.813	1.791	1.802	1.775
250	1.281	1.133	1.207	1.180
125	0.771	0.75	0.761	0.734
62.5	0.467	0.455	0.461	0.434
31.25	0.276	0.289	0.283	0.256
15.63	0.17	0.154	0.162	0.135
0	0.035	0.019	0.027	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										

删除[a]: **Sensitivity**

The lower limit of detection of mouse CCL2 is 1.96 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<6%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<9%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of mouse CCL2 into serum, repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 89% to 115%, with a mean recovery of 104%.

Linearity of dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of mouse CCL2 into serum. The linearity range is the ratio of the assay value to the expected value of mouse CCL2 in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
91
85-104

1:4
112
97-117

1:8
103
93-107

1:16
98
91-103

Sample values

The kit was applied to 30 normal mouse serum/plasma

B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

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Servicebio® Mouse IFN-beta ELISA Kit

Cat. #：GEM0018

Product Information

Product Name	Cat. No.	Spec.
Mouse IFN-beta ELISA Kit	GEM0018-48T	48T
	GEM0018-96T	96T

Product Description

Interferon (IFN) is a glycoprotein produced by humans or animals in response to viral infection or other IFN-inducing agents. It has various functions such as antiviral, antitumor and immune regulation. Currently, IFN is classified into three types: type I, type II and type III. IFN-beta, also known as fibroblast IFN, is a member of the type I interferon molecule family with a molecular weight of about 22 kDa, produced by fibroblasts and macrophages in response to viral infection and antigen stimulation. IFN-beta plays an important role in inducing non-specific antiviral infection, affecting cell proliferation and regulating immune responses. It has a broad-spectrum antiviral effect, but does not directly kill viruses. Instead, it binds to IFN receptors on the cell surface, upregulating and downregulating multiple genes via the STAT1 and STAT2 signaling pathways, inducing the production of antiviral proteins (AVPs) in cells and exerting an antiviral effect. The Mouse IFN-beta ELISA Kit uses a dual antibody sandwich enzyme-linked immunosorbent assay method to quantitatively detect IFN-beta in mouse serum, plasma, cell culture supernatant or other relevant fluids, and can simultaneously detect natural and recombinant mouse IFN-beta.

删除[a]: tissue homogenate, cell lysate,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GEM0018-48T	GEM0018-96T
GEM0018-1	Microplate	48T	96T
GEM0018-2	Standard	1 vial	2 vials
GEM0018-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm(Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Mouse IFN-beta Standard is obtained by redissolving the standard to a concentration of 4000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
      Mix the reconstituted standards well, add 500 µL of Diluent A to 500 µL of concentrated Mouse IFN-beta standard as the highest concentration S7 (2000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Diluent A. Pipette 500 µL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
      Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse IFN-beta standard as the highest concentration S7 (2000 pg/mL) for the

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

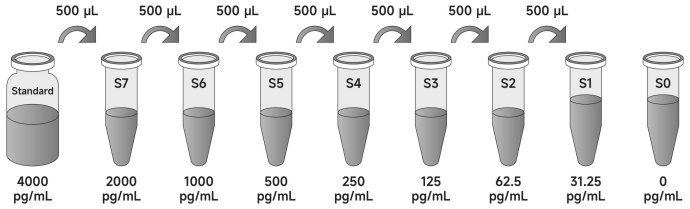
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 μL each of culture supernatant. Pipette 500 μL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 μL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 μL of standards of different concentrations to each well, add 100 μL of Diluent A to the blank wells, and 100 μL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 μL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Detection Antibody Addition:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 μL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.

5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

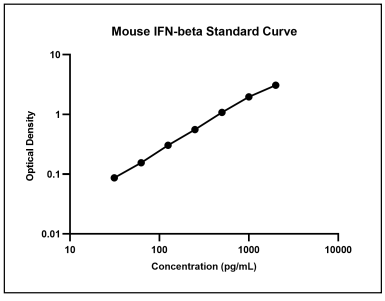
Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
2000	3.019	3.188	3.104	3.083
1000	1.95	2.034	1.992	1.972
500	1.076	1.126	1.101	1.081
250	0.561	0.592	0.577	0.556
125	0.293	0.356	0.325	0.304
62.50	0.167	0.185	0.176	0.156
31.25	0.102	0.113	0.108	0.087
0	0.018	0.023	0.021	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12

删除[a]: **Sensitivity**

The lower limit of detection of mouse IFN-beta is 8.72 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV (%) =(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<6%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<10%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of mouse IFN-beta into serum, repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 85% to 108%, with a mean recovery of 97%.

Linearity of dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of mouse IFN-beta into serum. The linearity range is the ratio of the assay value to the expected value of mouse IFN-beta in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
93
82-102

1:4
95
88-115

1:8
106
92-117

1:16
102
95-107

Sample values

A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Mouse IFN-beta ELISA Kit

Cat. #：GEM0018

Product Information

Product Name	Cat. No.	Spec.
Mouse IFN-beta ELISA Kit	GEM0018-48T	48T
	GEM0018-96T	96T

Product Description

Interferon (IFN) is a glycoprotein produced by humans or animals in response to viral infection or other IFN-inducing agents. It has various functions such as antiviral, antitumor and immune regulation. Currently, IFN is classified into three types: type I, type II and type III. IFN-beta, also known as fibroblast IFN, is a member of the type I interferon molecule family with a molecular weight of about 22 kDa, produced by fibroblasts and macrophages in response to viral infection and antigen stimulation. IFN-beta plays an important role in inducing non-specific antiviral infection, affecting cell proliferation and regulating immune responses. It has a broad-spectrum antiviral effect, but does not directly kill viruses. Instead, it binds to IFN receptors on the cell surface, upregulating and downregulating multiple genes via the STAT1 and STAT2 signaling pathways, inducing the production of antiviral proteins (AVPs) in cells and exerting an antiviral effect. The Mouse IFN-beta ELISA Kit uses a dual antibody sandwich enzyme-linked immunosorbent assay method to quantitatively detect IFN-beta in mouse serum, plasma, cell culture supernatant or other relevant fluids, and can simultaneously detect natural and recombinant mouse IFN-beta.

删除[a]: tissue homogenate, cell lysate,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GEM0018-48T	GEM0018-96T
GEM0018-1	Microplate	48T	96T
GEM0018-2	Standard	1 vial	2 vials
GEM0018-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm(Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Mouse IFN-beta Standard is obtained by redissolving the standard to a concentration of 4000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
      Mix the reconstituted standards well, add 500 µL of Diluent A to 500 µL of concentrated Mouse IFN-beta standard as the highest concentration S7 (2000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Diluent A. Pipette 500 µL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
      Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse IFN-beta standard as the highest concentration S7 (2000 pg/mL) for the

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

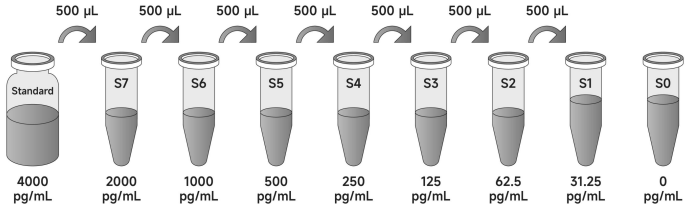
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 μL each of culture supernatant. Pipette 500 μL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 μL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 μL of standards of different concentrations to each well, add 100 μL of Diluent A to the blank wells, and 100 μL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 μL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Detection Antibody Addition:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 μL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.

5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

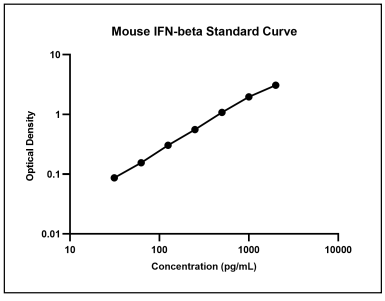
Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
2000	3.019	3.188	3.104	3.083
1000	1.95	2.034	1.992	1.972
500	1.076	1.126	1.101	1.081
250	0.561	0.592	0.577	0.556
125	0.293	0.356	0.325	0.304
62.50	0.167	0.185	0.176	0.156
31.25	0.102	0.113	0.108	0.087
0	0.018	0.023	0.021	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12

删除[a]: **Sensitivity**

The lower limit of detection of mouse IFN-beta is 8.72 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV (%) =(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<6%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<10%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of mouse IFN-beta into serum, repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 85% to 108%, with a mean recovery of 97%.

Linearity of dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of mouse IFN-beta into serum. The linearity range is the ratio of the assay value to the expected value of mouse IFN-beta in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
93
82-102

1:4
95
88-115

1:8
106
92-117

1:16
102
95-107

Sample values

samples, all

A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Mouse MMP-9 ELISA Kit

Cat. #: GEM0021

Product Information

Product Name	Cat. No.	Spec.
Mouse MMP-9 ELISA Kit	GEM0021-48T	48T
	GEM0021-96T	96T

Product Description/Introduction

Matrix metalloproteinase 9 (MMP-9), also known as 92 KDa type IV collagenase, 92 KDa gelatinase, or gelatinase B, belongs to the zinc-metalloproteinase family. MMP-9 plays an important role in vascularity and neovascularisation, it is significantly up-regulated in human respiratory epithelial healing and has been found to be associated with a number of pathological processes such as cancer, immune and cardiovascular diseases, rheumatoid arthritis, etc. MMP-9 is involved in normal physiological processes (e.g., embryonic development, reproduction, skeletal development, wound healing, cell migration, learning and memory) and pathological processes (e.g. arthritis, cerebral haemorrhage and tumour metastasis) in extracellular matrix degradation. Mouse MMP-9 Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates MMP-9 in Mouse serum, plasma or cell culture medium. The assay will exclusively recognize both natural and recombinant Mouse MMP-9.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product components

Component Number	Component	GEM0021-48T	GEM0021-96T
GEM0021-1	Microplate	48T	96T
GEM0021-2	Standard	1 vial	2 vials
GEM0021-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample collection and storage instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

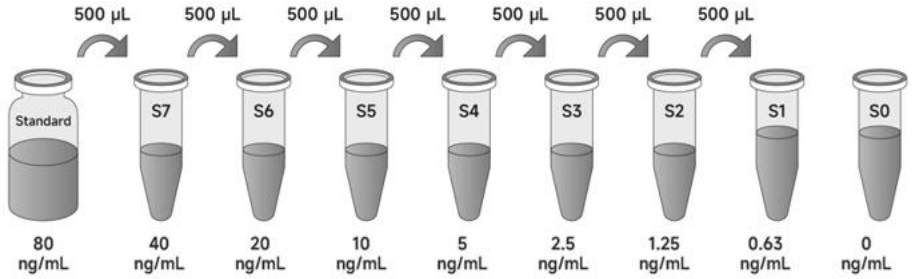
1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Mouse MMP-9 Standard is obtained by redissolving the standard to a concentration of 80 ng/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.

a) Preparation of standard curves for serum/plasma:

Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated Mouse MMP-9 standard as the highest concentration S7 (40 ng/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (40 ng/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

b) Preparation of standard curve of cell culture supernatant sample:

Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse MMP-9 standard as the highest concentration S7 (40 ng/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (40 ng/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol/Procedures

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 µL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 µL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled

for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).

8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

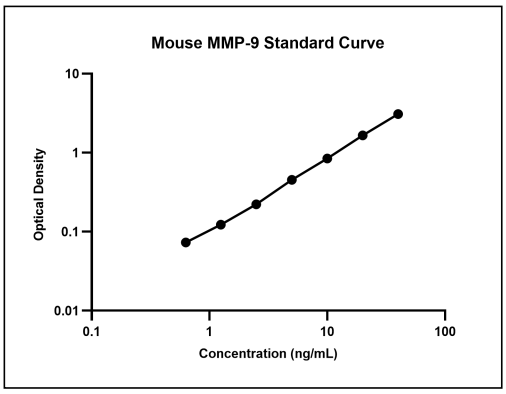
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

ng/mL	OD		Average	Corrected
40	3.194	3.090	3.142	3.079
20	1.695	1.747	1.721	1.658
10	0.836	0.979	0.908	0.845
5	0.462	0.572	0.517	0.454
2.5	0.275	0.295	0.285	0.222
1.25	0.174	0.197	0.186	0.123
0.63	0.127	0.145	0.136	0.073
0	0.056	0.069	0.063	



Test protocol summary

1. Prepare standards, reagents and samples according to instructions before the experiment.

2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.

3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.

4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.

5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.

6. Add 50 µL of stop solution to all wells.

7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Mouse MMP-9 ELISA Kit

Cat. #: GEM0021

Product Information

Product Name	Cat. No.	Spec.
Mouse MMP-9 ELISA Kit	GEM0021-48T	48T
	GEM0021-96T	96T

Product Description/Introduction

Matrix metalloproteinase 9 (MMP-9), also known as 92 KDa type IV collagenase, 92 KDa gelatinase, or gelatinase B, belongs to the zinc-metalloproteinase family. MMP-9 plays an important role in vascularity and neovascularisation, it is significantly up-regulated in human respiratory epithelial healing and has been found to be associated with a number of pathological processes such as cancer, immune and cardiovascular diseases, rheumatoid arthritis, etc. MMP-9 is involved in normal physiological processes (e.g., embryonic development, reproduction, skeletal development, wound healing, cell migration, learning and memory) and pathological processes (e.g. arthritis, cerebral haemorrhage and tumour metastasis) in extracellular matrix degradation. Mouse MMP-9 Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates MMP-9 in Mouse serum, plasma or cell culture medium. The assay will exclusively recognize both natural and recombinant Mouse MMP-9.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product components

Component Number	Component	GEM0021-48T	GEM0021-96T
GEM0021-1	Microplate	48T	96T
GEM0021-2	Standard	1 vial	2 vials
GEM0021-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

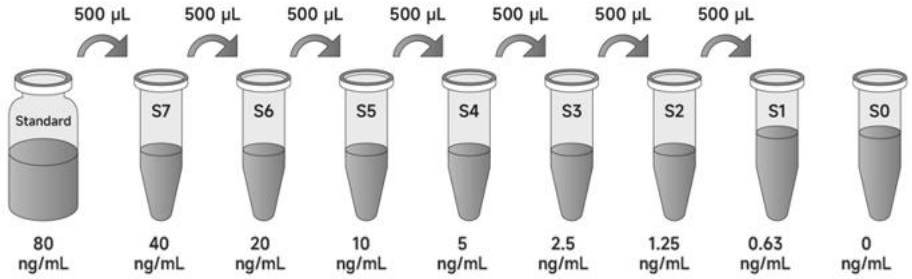
1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Mouse MMP-9 Standard is obtained by redissolving the standard to a concentration of 80 ng/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.

a) Preparation of standard curves for serum/plasma:

Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated Mouse MMP-9 standard as the highest concentration S7 (40 ng/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (40 ng/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

b) Preparation of standard curve of cell culture supernatant sample:

Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse MMP-9 standard as the highest concentration S7 (40 ng/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (40 ng/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol/Procedures

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 µL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 µL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled

for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).

8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

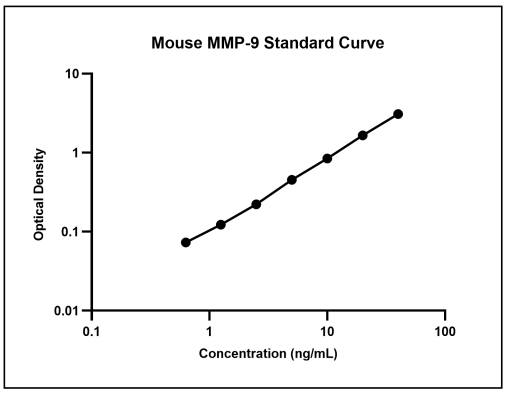
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

ng/mL	OD		Average	Corrected
40	3.194	3.090	3.142	3.079
20	1.695	1.747	1.721	1.658
10	0.836	0.979	0.908	0.845
5	0.462	0.572	0.517	0.454
2.5	0.275	0.295	0.285	0.222
1.25	0.174	0.197	0.186	0.123
0.63	0.127	0.145	0.136	0.073
0	0.056	0.069	0.063	



Test protocol summary

1. Prepare standards, reagents and samples according to instructions before the experiment.

2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.

3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.

4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.

5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.

6. Add 50 µL of stop solution to all wells.

7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Mouse VEGF ELISA Kit

Cat. #: GEM0022

Product Information

Product Name	Cat. No.	Spec.
Mouse VEGF ELISA Kit	GEM0022-48T	48T
	GEM0022-96T	96T

Product Description/Introduction

Vascular Endothelial Growth Factor (VEGF), originally named vascular permeability factor (VPF), is an important regulator of angiogenesis and vasculogenesis. Tumour neoangiogenesis is carried out and completed under the regulation of vascular endothelial growth factor. The active degree of tumour angiogenesis is an important factor affecting the proliferation of tumour cells, and VEGF is one of the most important promoters of tumour angiogenesis, so it can reflect the occurrence, development and regression of tumours. By measuring the level of VEGF in serum, it can be used for early screening of tumours, observation of the efficacy of treatment and recurrence monitoring of tumour patients, and auxiliary diagnosis, monitoring and evaluation of diseases related to angiogenesis.

Mouse VEGF Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates VEGF in Mouse serum, plasma, or cell culture medium. The assay will exclusively recognize both natural and recombinant Mouse VEGF.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product components

Component Number	Component	GEM0022-48T	GEM0022-96T
GEM0022-1	Microplate	48T	96T
GEM0022-2	Standard	1 vial	2 vials
GEM0022-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample collection and storage instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

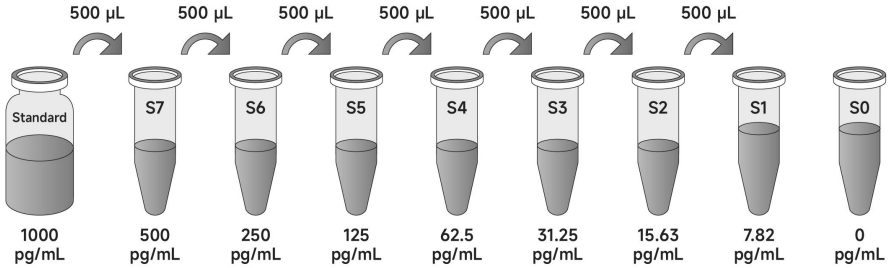
1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Mouse VEGF Standard is obtained by redissolving the standard to a concentration of 1000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.

a) Preparation of standard curves for serum/plasma:

Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated Mouse VEGF standard as the highest concentration S7 (500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

b) Preparation of standard curve of cell culture supernatant sample:

Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse VEGF standard as the highest concentration S7 (500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol/Procedures

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 µL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.

7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation

coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

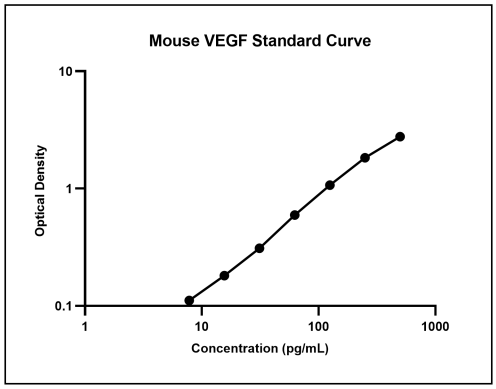
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
500	2.833	2.748	2.791	2.770
250	1.873	1.833	1.853	1.833
125	1.071	1.107	1.089	1.069
62.5	0.605	0.624	0.615	0.594
31.25	0.316	0.344	0.330	0.310
15.63	0.210	0.192	0.201	0.181
7.82	0.120	0.142	0.131	0.111
0	0.016	0.025	0.021	



Test protocol summary

1. Prepare standards, reagents and samples according to instructions before the experiment.

↓

2. Add 100 μL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.

↓

3. Wash 5 times and pat dry, add 100 μL of detection antibody working solution to all wells and shake for 1 hour at room temperature.

↓

4. Wash 5 times and pat dry, add 100 μL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.

↓

5. Wash 5 times and pat dry, add 90 μL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.

↓

6. Add 50 μL of stop solution to all wells.

↓

7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Mouse VEGF ELISA Kit

Cat. #: GEM0022

Product Information

Product Name	Cat. No.	Spec.
Mouse VEGF ELISA Kit	GEM0022-48T	48T
	GEM0022-96T	96T

Product Description/Introduction

Vascular Endothelial Growth Factor (VEGF), originally named vascular permeability factor (VPF), is an important regulator of angiogenesis and vasculogenesis. Tumour neoangiogenesis is carried out and completed under the regulation of vascular endothelial growth factor. The active degree of tumour angiogenesis is an important factor affecting the proliferation of tumour cells, and VEGF is one of the most important promoters of tumour angiogenesis, so it can reflect the occurrence, development and regression of tumours. By measuring the level of VEGF in serum, it can be used for early screening of tumours, observation of the efficacy of treatment and recurrence monitoring of tumour patients, and auxiliary diagnosis, monitoring and evaluation of diseases related to angiogenesis.

Mouse VEGF Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates VEGF in Mouse serum, plasma, or cell culture medium. The assay will exclusively recognize both natural and recombinant Mouse VEGF.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product components

Component Number	Component	GEM0022-48T	GEM0022-96T
GEM0022-1	Microplate	48T	96T
GEM0022-2	Standard	1 vial	2 vials
GEM0022-3	Detection Antibody	60 µL	120 µL
G0023	SA-HRP	60 µL	120 µL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample collection and storage instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

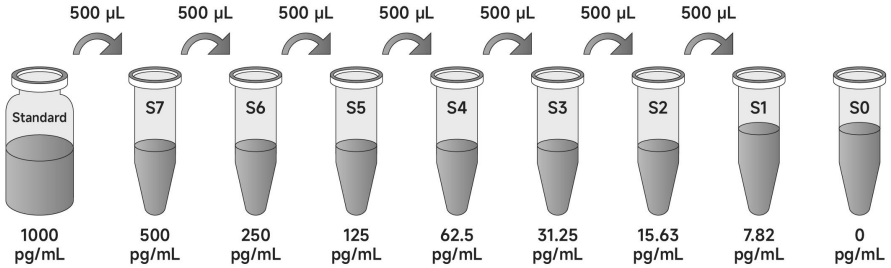
1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Mouse VEGF Standard is obtained by redissolving the standard to a concentration of 1000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.

a) Preparation of standard curves for serum/plasma:

Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated Mouse VEGF standard as the highest concentration S7 (500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

b) Preparation of standard curve of cell culture supernatant sample:

Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse VEGF standard as the highest concentration S7 (500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol/Procedures

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 µL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.

7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation

coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
500	2.833	2.748	2.791	2.770
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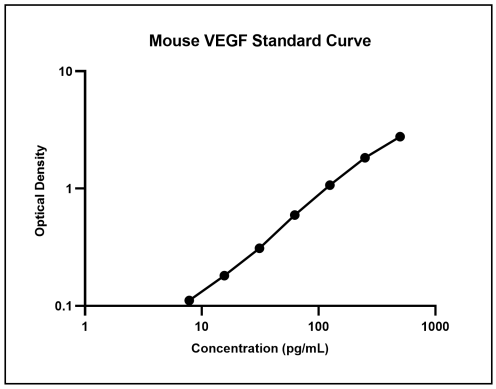
125	1.071	1.107	1.089	1.069
62.5	0.605	0.624	0.615	0.594
31.25	0.316	0.344	0.330	0.310
15.63	0.210	0.192	0.201	0.181
7.82	0.120	0.142	0.131	0.111
0	0.016	0.025	0.021	



Test protocol summary

1. Prepare standards, reagents and samples according to instructions before the experiment.

↓

2. Add 100 μL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.

↓

3. Wash 5 times and pat dry, add 100 μL of detection antibody working solution to all wells and shake for 1 hour at room temperature.

↓

4. Wash 5 times and pat dry, add 100 μL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.

↓

5. Wash 5 times and pat dry, add 90 μL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.

↓

6. Add 50 μL of stop solution to all wells.

↓

7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Mouse CXCL1/KC ELISA Kit

Cat. #：GEM0024

Product Information

Product Name	Cat. No.	Spec.
Mouse CXCL1/KC ELISA Kit	GEM0024-48T	48T
	GEM0024-96T	96T

Product Description

The chemokine ligand 1 of the C-X-C motif, also known as CXCL1, KC, GRO-α, NAP-3, or MGSA, belongs to the CXC chemokine subfamily. CXCL1 serves as a growth factor for melanoma cells and a chemoattractant for neutrophils. It is an effective neutrophil activator and attractant, also exhibiting activity towards eosinophils. CXCL1 is expressed by macrophages, neutrophils, and epithelial cells, and possesses neutrophil chemotactic activity. CXCL1 is involved in spinal cord development by suppressing the migration of oligodendrocyte precursors, and plays roles in angiogenesis, inflammation, wound healing, and tumorigenesis processes. The Mouse CXCL1/KC ELISA Kit utilizes dual antibody sandwich enzyme-linked immunosorbent assay technology to quantitatively detect CXCL1/KC in mouse serum, plasma, cell culture supernatants, or other related fluids. It can simultaneously detect natural and recombinant mouse CXCL1/KC.

删除[a]: tissue homogenates, cell lysates,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GEM0024-48T	GEM0024-96T
GEM0024-1	Microplate	48T	96T
GEM0024-2	Standard	1 vial	2 vials
GEM0024-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm(Refer

to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Mouse CXCL1 Standard is obtained by redissolving the standard to a concentration of 8000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
      Mix the reconstituted standards well, add 500 µL of Diluent A to 500 µL of concentrated Mouse CXCL1 standard as the highest concentration S7 (4000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Diluent A. Pipette 500 µL of S7 (4000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
      Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse CXCL1 standard as the highest concentration S7 (4000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

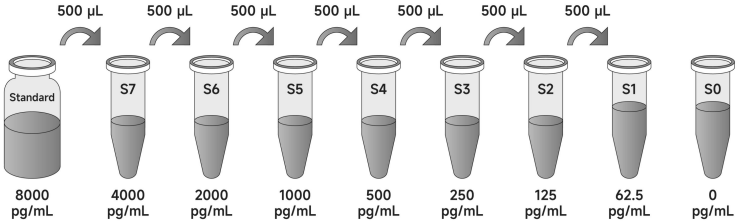
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

each of culture supernatant. Pipette 500 µL of S7 (4000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Detection Antibody Addition:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.

5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

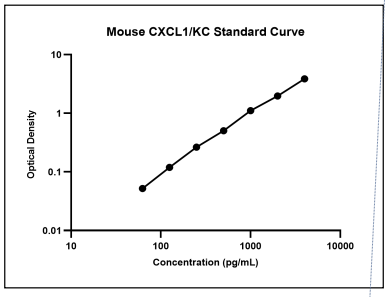
Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
4000	3.910	3.890	3.900	3.846
2000	2.108	1.930	2.019	1.965
1000	1.123	1.191	1.157	1.103
500	0.559	0.552	0.556	0.502
250	0.322	0.314	0.318	0.264
125	0.169	0.176	0.173	0.119
62.5	0.104	0.108	0.106	0.052
0	0.058	0.049	0.054	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

删除[a]: **Sensitivity**

The lower limit of detection of mouse CXCL1 is 4.72 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV (%) =(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<6%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<8%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of mouse CXCL1 into serum, repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 86% to 101%, with a mean recovery of 95%.

Linearity of dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of mouse CXCL1 into serum. The linearity range is the ratio of the assay value to the expected value of mouse CXCL1 in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
99
95-104

1:4
93
86-101

1:8
105
92-112

1:16
95
89-109

Sample values

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat IL-6 ELISA Kit

Cat. #: GER0001

Product Information

Product Name	Cat. No.	Spec.
Rat IL-6 ELISA Kit	GER0001-48T	48T
	GER0001-96T	96T

Product Description

Interleukin 6 (IL-6) is a multifunctional 26 kD protein originally discovered in the medium of RNA-stimulated fibroblastoid cells. IL-6 appears to be directly involved in the responses that occur after infection and cellular injury, and it may prove to be important as IL-1 and TNF-a in regulating the acute phase response. IL-6 is reported to be produced by fibroblasts, activated T cells, activated monocytes or macrophages and endothelial cells. Primarily produced at sites of acute and chronic inflammation, IL-6 is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. The functioning of IL-6 is implicated in a wide variety of inflammation-associated disease states including diabetes mellitus and systemic juvenile rheumatoid arthritis. Rat IL-6 ELISA Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitative measurement of rat IL-6 in serum, plasma, cell culture supernatants or other relevant fluids, and for the simultaneous detection of natural and recombinant rat IL-6.

删除[a]: tissue homogenates, cell lysates,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0001-48T	GER0001-96T
GER0001-1	Microplate	48T	96T
GER0001-2	Standard	1 vial	2 vials
GER0001-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum**: Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma**: Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat IL-6 Standard is obtained by redissolving the standard to a concentration of 2000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma/tissue homogenate/cell lysate:
   Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated rat IL-6 standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

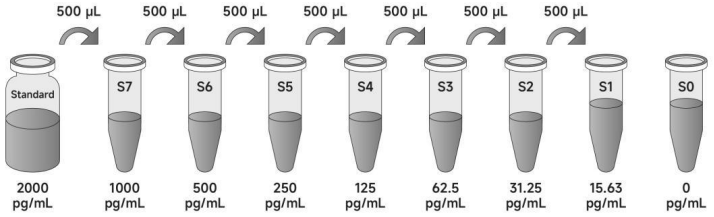
删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

b) Preparation of standard curve of cell culture supernatant sample:
Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated rat IL-6 standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.
   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a

new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.

4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

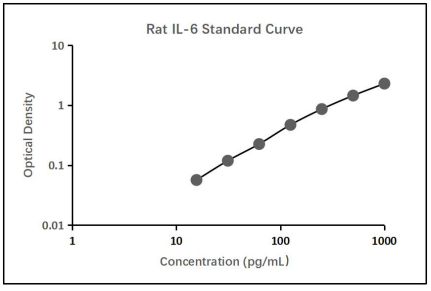
1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to

remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
1000	2.333	2.353	2.343	2.297
500	1.46	1.531	1.496	1.450
250	0.883	0.933	0.908	0.862
125	0.514	0.519	0.517	0.471
62.5	0.266	0.276	0.271	0.225
31.25	0.164	0.165	0.165	0.119
15.63	0.099	0.106	0.103	0.057
0	0.049	0.043	0.046	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells.
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[a]: **Sensitivity**

The lower limit of detection of rat IL-6 is 1.45 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<8%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<10%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IL-6 into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 84% to 107%, with a mean recovery of 98%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IL-6 into serum. The linearity range is the ratio of the assay value to the expected value of rat IL-6 in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
102
90-110

1:4
100
95-108

1:8
97
90-106

1:16
95
89-102

Sample values

The kit was applied to 30 normal rat serum samples and all

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

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Servicebio® Rat IL-6 ELISA Kit

Cat. # : GER0001

Product Information

Product Name	Cat. No.	Spec.
Rat IL-6 ELISA Kit	GER0001-48T	48T
	GER0001-96T	96T

Product Description

Interleukin 6 (IL-6) is a multifunctional 26 kD protein originally discovered in the medium of RNA-stimulated fibroblastoid cells. IL-6 appears to be directly involved in the responses that occur after infection and cellular injury, and it may prove to be important as IL-1 and TNF-a in regulating the acute phase response. IL-6 is reported to be produced by fibroblasts, activated T cells, activated monocytes or macrophages and endothelial cells. Primarily produced at sites of acute and chronic inflammation, IL-6 is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. The functioning of IL-6 is implicated in a wide variety of inflammation-associated disease states including diabetes mellitus and systemic juvenile rheumatoid arthritis. Rat IL-6 ELISA Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitative measurement of rat IL-6 in serum, plasma, cell culture supernatants or other relevant fluids, and for the simultaneous detection of natural and recombinant rat IL-6.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0001-48T	GER0001-96T
GER0001-1	Microplate	48T	96T
GER0001-2	Standard	1 vial	2 vials
GER0001-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum**: Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma**: Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat IL-6 Standard is obtained by redissolving the standard to a concentration of 2000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma/tissue homogenate/cell lysate:
   Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated rat IL-6 standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

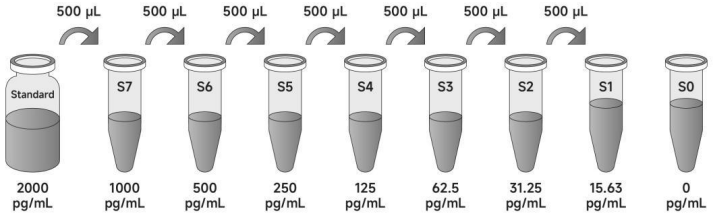
删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

b) Preparation of standard curve of cell culture supernatant sample:
Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated rat IL-6 standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.
   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a

new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.

4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

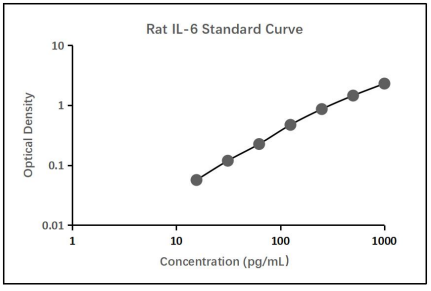
1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to

remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
1000	2.333	2.353	2.343	2.297
500	1.46	1.531	1.496	1.450
250	0.883	0.933	0.908	0.862
125	0.514	0.519	0.517	0.471
62.5	0.266	0.276	0.271	0.225
31.25	0.164	0.165	0.165	0.119
15.63	0.099	0.106	0.103	0.057
0	0.049	0.043	0.046	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells.
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[a]: **Sensitivity**

The lower limit of detection of rat IL-6 is 1.45 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<8%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<10%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IL-6 into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 84% to 107%, with a mean recovery of 98%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IL-6 into serum. The linearity range is the ratio of the assay value to the expected value of rat IL-6 in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
102
90-110

1:4
100
95-108

1:8
97
90-106

1:16
95
89-102

Sample values

The kit was applied to 30 normal rat serum samples and all

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

For Research Use Only!

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Servicebio® Rat IL-1 beta ELISA Kit

Cat. #：GER0002

Product Information

Product Name	Cat. No.	Spec.
Rat IL-1 beta ELISA Kit	GER0002-48T	48T
	GER0002-96T	96T

Product Description/Introduction

Interleukin-1 beta (IL-1 beta) is a proinflammatory cytokine expressed by monocytes, macrophages, and dendritic cells. IL-1 beta is synthesized in response to inflammatory stimuli as a 31 kDa inactive pro-form that accumulates in the cytosol. Cleavage of pro-IL-1 beta into the active 17 kDa protein requires the activation of inflammasomes, which are multi-protein complexes that respond to pathogens, stress conditions, and other danger signals. The Rat Interleukin-1beta (IL-1β) is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates IL-1β in rat serum, or cell culture medium. The assay will exclusively recognize both natural and recombinant rat IL-1β.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0002-48T	GER0002-96T
GER0002-1	Microplate	48T	96T
GER0002-2	Standard	1 vial	2 vials
GER0002-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.

2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat IL-1 beta Standard is obtained by redissolving the standard to a concentration of 5000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
   Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated rat IL-1 beta standard as the highest concentration S7 (2500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (2500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
   Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated rat IL-1 beta standard as the highest concentration S7 (2500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (2500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.

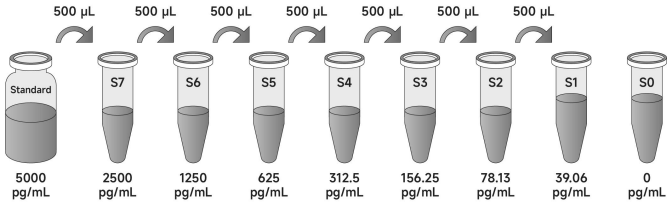
删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol/Procedures

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Detection Antibody Addition:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate Washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 µL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 µL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled

for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)

8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results analysis

1. **Results Calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

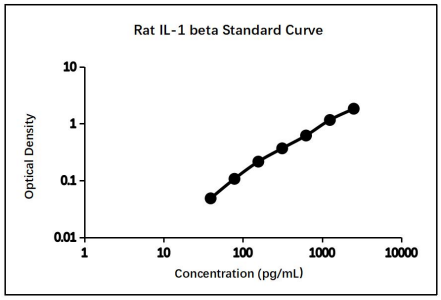
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
2500	1.831	1.927	1.879	1.834
1250	1.205	1.207	1.206	1.161
625	0.661	0.666	0.664	0.619
312.5	0.414	0.412	0.413	0.368
156.25	0.255	0.265	0.260	0.215
78.13	0.151	0.153	0.152	0.107
39.06	0.092	0.095	0.094	0.049
0	0.047	0.042	0.045	



Test protocol summary

1. Prepare standards, reagents and samples according to instructions before the experiment.

2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.

3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.

4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.

5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.

6. Add 50 µL of stop solution to all wells

7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[a]: **Sensitivity**

The lower limit of detection of rat IL-1 beta is 2.99 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<4%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<7%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IL-1 beta into serum, repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 88% to 101%, with a mean recovery of 94%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IL-1 beta into serum. The linearity range is the ratio of the assay value to the expected value of rat IL-1 beta in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
103
93-109

1:4
91
81-98

1:8
110
98-115

1:16
101
94-106

Sample values

The kit was applied to 30 normal rat serum samples and all

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

For Research Use Only. Not for use in diagnostic procedures!

Servicebio® Rat IL-1 beta ELISA Kit

Cat. #：GER0002

Product Information

Product Name	Cat. No.	Spec.
Rat IL-1 beta ELISA Kit	GER0002-48T	48T
	GER0002-96T	96T

Product Description/Introduction

Interleukin-1 beta (IL-1 beta) is a proinflammatory cytokine expressed by monocytes, macrophages, and dendritic cells. IL-1 beta is synthesized in response to inflammatory stimuli as a 31 kDa inactive pro-form that accumulates in the cytosol. Cleavage of pro-IL-1 beta into the active 17 kDa protein requires the activation of inflammasomes, which are multi-protein complexes that respond to pathogens, stress conditions, and other danger signals. The Rat Interleukin-1beta (IL-1β) is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates IL-1β in rat serum, or cell culture medium. The assay will exclusively recognize both natural and recombinant rat IL-1β.

删除[a]: plasma, tissue homogenates, cell lysates,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0002-48T	GER0002-96T
GER0002-1	Microplate	48T	96T
GER0002-2	Standard	1 vial	2 vials
GER0002-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.

2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat IL-1 beta Standard is obtained by redissolving the standard to a concentration of 5000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
   Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated rat IL-1 beta standard as the highest concentration S7 (2500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (2500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
   Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated rat IL-1 beta standard as the highest concentration S7 (2500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (2500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.

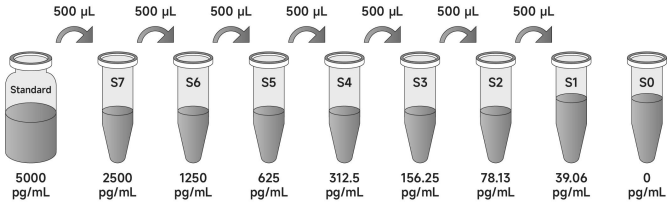
删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol/Procedures

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Detection Antibody Addition:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate Washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 µL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 µL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled

for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)

8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results analysis

1. **Results Calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

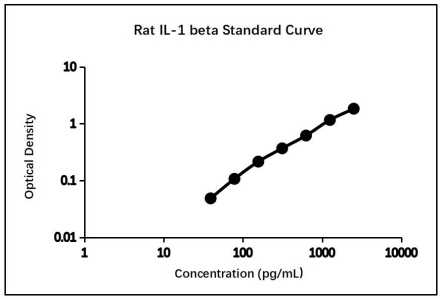
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
2500	1.831	1.927	1.879	1.834
1250	1.205	1.207	1.206	1.161
625	0.661	0.666	0.664	0.619
312.5	0.414	0.412	0.413	0.368
156.25	0.255	0.265	0.260	0.215
78.13	0.151	0.153	0.152	0.107
39.06	0.092	0.095	0.094	0.049
0	0.047	0.042	0.045	



Test protocol summary

1. Prepare standards, reagents and samples according to instructions before the experiment.

2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.

3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.

4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.

5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.

6. Add 50 µL of stop solution to all wells

7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[a]: **Sensitivity**

The lower limit of detection of rat IL-1 beta is 2.99 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<4%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<7%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IL-1 beta into serum, repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 88% to 101%, with a mean recovery of 94%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IL-1 beta into serum. The linearity range is the ratio of the assay value to the expected value of rat IL-1 beta in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
103
93-109

1:4
91
81-98

1:8
110
98-115

1:16
101
94-106

Sample values

The kit was applied to 30 normal rat serum samples and all

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat IL-1 beta ELISA Kit

Cat. #：GER0002

Product Information

Product Name	Cat. No.	Spec.
Rat IL-1 beta ELISA Kit	GER0002-48T	48T
	GER0002-96T	96T

Product Description/Introduction

Interleukin-1 beta (IL-1 beta) is a proinflammatory cytokine expressed by monocytes, macrophages, and dendritic cells. IL-1 beta is synthesized in response to inflammatory stimuli as a 31 kDa inactive pro-form that accumulates in the cytosol. Cleavage of pro-IL-1 beta into the active 17 kDa protein requires the activation of inflammasomes, which are multi-protein complexes that respond to pathogens, stress conditions, and other danger signals. The Rat Interleukin-1beta (IL-1β) is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates IL-1β in rat serum, or cell culture medium. The assay will exclusively recognize both natural and recombinant rat IL-1β.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0002-48T	GER0002-96T
GER0002-1	Microplate	48T	96T
GER0002-2	Standard	1 vial	2 vials
GER0002-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.

2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat IL-1 beta Standard is obtained by redissolving the standard to a concentration of 5000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
   Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated rat IL-1 beta standard as the highest concentration S7 (2500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (2500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
   Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated rat IL-1 beta standard as the highest concentration S7 (2500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (2500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.

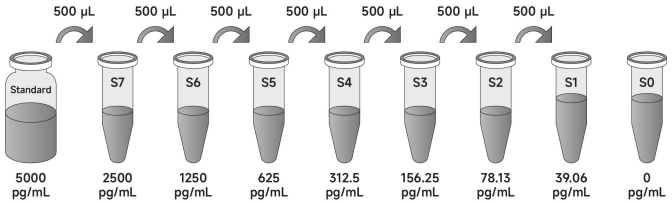
删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol/Procedures

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Detection Antibody Addition:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate Washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 µL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 µL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled

for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)

8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
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8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results analysis

1. **Results Calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
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39.06	0.092	0.095	0.094	0.049
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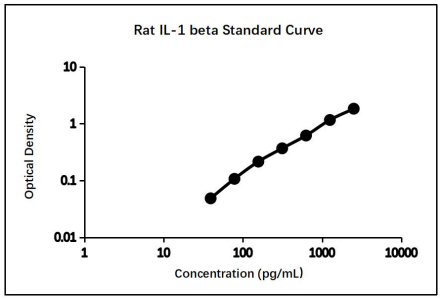


Test protocol summary

1. Prepare standards, reagents and samples according to instructions before the experiment.

2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.

3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.

4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.

5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.

6. Add 50 µL of stop solution to all wells

7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[a]: **Sensitivity**

The lower limit of detection of rat IL-1 beta is 2.99 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<4%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<7%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IL-1 beta into serum, repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 88% to 101%, with a mean recovery of 94%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IL-1 beta into serum. The linearity range is the ratio of the assay value to the expected value of rat IL-1 beta in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
103
93-109

1:4
91
81-98

1:8
110
98-115

1:16
101
94-106

Sample values

The kit was applied to 30 normal rat serum samples and all

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat IL-1 beta ELISA Kit

Cat. #：GER0002

Product Information

Product Name	Cat. No.	Spec.
Rat IL-1 beta ELISA Kit	GER0002-48T	48T
	GER0002-96T	96T

Product Description/Introduction

Interleukin-1 beta (IL-1 beta) is a proinflammatory cytokine expressed by monocytes, macrophages, and dendritic cells. IL-1 beta is synthesized in response to inflammatory stimuli as a 31 kDa inactive pro-form that accumulates in the cytosol. Cleavage of pro-IL-1 beta into the active 17 kDa protein requires the activation of inflammasomes, which are multi-protein complexes that respond to pathogens, stress conditions, and other danger signals. The Rat Interleukin-1beta (IL-1β) is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates IL-1β in rat serum, or cell culture medium. The assay will exclusively recognize both natural and recombinant rat IL-1β.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0002-48T	GER0002-96T
GER0002-1	Microplate	48T	96T
GER0002-2	Standard	1 vial	2 vials
GER0002-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.

2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat IL-1 beta Standard is obtained by redissolving the standard to a concentration of 5000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
   Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated rat IL-1 beta standard as the highest concentration S7 (2500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (2500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
   Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated rat IL-1 beta standard as the highest concentration S7 (2500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (2500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.

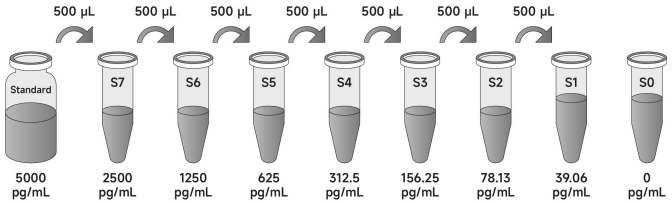
删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol/Procedures

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Detection Antibody Addition:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate Washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 µL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 µL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled

for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)

8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results analysis

1. **Results Calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

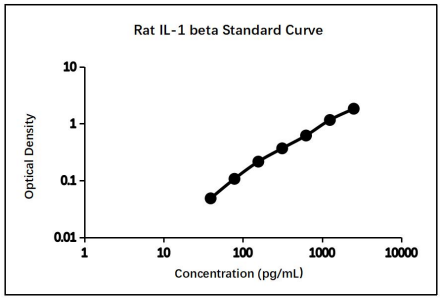
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
2500	1.831	1.927	1.879	1.834
1250	1.205	1.207	1.206	1.161
625	0.661	0.666	0.664	0.619
312.5	0.414	0.412	0.413	0.368
156.25	0.255	0.265	0.260	0.215
78.13	0.151	0.153	0.152	0.107
39.06	0.092	0.095	0.094	0.049
0	0.047	0.042	0.045	



Test protocol summary

1. Prepare standards, reagents and samples according to instructions before the experiment.

2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.

3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.

4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.

5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.

6. Add 50 µL of stop solution to all wells

7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[a]: **Sensitivity**

The lower limit of detection of rat IL-1 beta is 2.99 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<4%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<7%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IL-1 beta into serum, repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 88% to 101%, with a mean recovery of 94%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IL-1 beta into serum. The linearity range is the ratio of the assay value to the expected value of rat IL-1 beta in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
103
93-109

1:4
91
81-98

1:8
110
98-115

1:16
101
94-106

Sample values

The kit was applied to 30 normal rat serum samples and all

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat IL-1 beta ELISA Kit

Cat. #：GER0002

Product Information

Product Name	Cat. No.	Spec.
Rat IL-1 beta ELISA Kit	GER0002-48T	48T
	GER0002-96T	96T

Product Description/Introduction

Interleukin-1 beta (IL-1 beta) is a proinflammatory cytokine expressed by monocytes, macrophages, and dendritic cells. IL-1 beta is synthesized in response to inflammatory stimuli as a 31 kDa inactive pro-form that accumulates in the cytosol. Cleavage of pro-IL-1 beta into the active 17 kDa protein requires the activation of inflammasomes, which are multi-protein complexes that respond to pathogens, stress conditions, and other danger signals. The Rat Interleukin-1beta (IL-1β) is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates IL-1β in rat serum, or cell culture medium. The assay will exclusively recognize both natural and recombinant rat IL-1β.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0002-48T	GER0002-96T
GER0002-1	Microplate	48T	96T
GER0002-2	Standard	1 vial	2 vials
GER0002-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat IL-1 beta Standard is obtained by redissolving the standard to a concentration of 5000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
   Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated rat IL-1 beta standard as the highest concentration S7 (2500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (2500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
   Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated rat IL-1 beta standard as the highest concentration S7 (2500 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (2500 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.

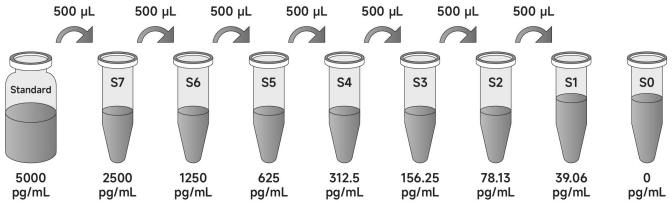
删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol/Procedures

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Detection Antibody Addition:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate Washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 µL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 µL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled

for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient.)

8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results analysis

1. **Results Calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
2500	1.831	1.927	1.879	1.834
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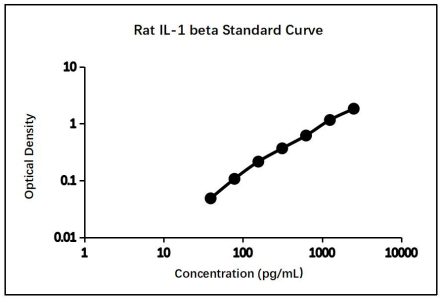
78.13	0.151	0.153	0.152	0.107
39.06	0.092	0.095	0.094	0.049
0	0.047	0.042	0.045	



Test protocol summary

1. Prepare standards, reagents and samples according to instructions before the experiment.

2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.

3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.

4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.

5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.

6. Add 50 µL of stop solution to all wells

7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[a]: **Sensitivity**

The lower limit of detection of rat IL-1 beta is 2.99 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<4%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<7%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IL-1 beta into serum, repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 88% to 101%, with a mean recovery of 94%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IL-1 beta into serum. The linearity range is the ratio of the assay value to the expected value of rat IL-1 beta in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
103
93-109

1:4
91
81-98

1:8
110
98-115

1:16
101
94-106

Sample values

The kit was applied to 30 normal rat serum samples and all

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

For Research Use Only. Not for use in diagnostic procedures!

Servicebio® Rat IL-10 ELISA Kit

Cat. #: GER0003

Product Information

Product Name	Cat. No.	Spec.
Rat IL-10 ELISA Kit	GER0003-48T	48T
	GER0003-96T	96T

Product Description

Interleukin 10 (IL-10, CSIF) is an anti-inflammatory cytokine mainly produced by macrophages and Th2 cells. The reported biological activities of IL-10, which maybe interrelated, include inhibition of macrophage-mediated cytokine synthesis, suppression of the delayed-type hyper-sensitivity response, and stimulation of the Th2 cell response which results in elevated antibody production. IL-10 functions by inhibiting pro-inflammatory cytokines made by macrophages and regulatory T cells including IFN-gamma, TNF-alpha, IL-2, and IL-3, IL-4, and GM-CSF. IL-10 is also known to suppress antigen presentation on antigen presenting cells, enhances B cell survival, proliferation, and antibody production. Rat IL-10 ELISA Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates IL-10 in rat serum, plasma, or cell culture medium. The assay will exclusively recognize both natural and recombinant rat IL-10.

删除[a]: tissue homogenates, cell lysates,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0003-48T	GER0003-96T
GER0003-1	Microplate	48T	96T
GER0003-2	Standard	1 vial	2 vials
GER0003-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer

to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum**: Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma**: Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat IL-10 Standard is obtained by redissolving the standard to a concentration of 4000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
   Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated rat IL-10 standard as the highest concentration S7 (2000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

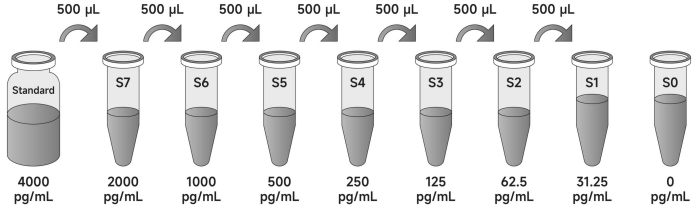
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20℃ to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated rat IL-10 standard as the highest concentration S7 (2000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a

new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.

4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

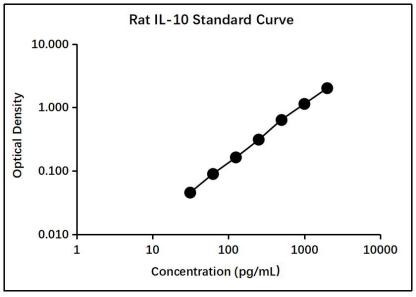
1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to

remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
2000	2.508	2.624	2.566	2.542
1000	1.321	1.411	1.366	1.342
500	0.815	0.901	0.858	0.834
250	0.48	0.512	0.496	0.472
125	0.35	0.337	0.344	0.319
62.50	0.261	0.246	0.254	0.229
31.25	0.181	0.173	0.177	0.153
0	0.076	0.068	0.072	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells.
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[a]: **Sensitivity**

The lower limit of detection of rat IL-10 is 6.08 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<8%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<11%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IL-10 into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 87% to 102%, with a mean recovery of 96%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IL-10 into serum. The linearity range is the ratio of the assay value to the expected value of rat IL-10 in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
89
83-95

1:4
95
91-102

1:8
98
94-101

1:16
102
96-108

Sample values

The kit was applied to 30 normal rat serum samples and all

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat IL-10 ELISA Kit

Cat. #: GER0003

Product Information

Product Name	Cat. No.	Spec.
Rat IL-10 ELISA Kit	GER0003-48T	48T
	GER0003-96T	96T

Product Description

Interleukin 10 (IL-10, CSIF) is an anti-inflammatory cytokine mainly produced by macrophages and Th2 cells. The reported biological activities of IL-10, which maybe interrelated, include inhibition of macrophage-mediated cytokine synthesis, suppression of the delayed-type hyper-sensitivity response, and stimulation of the Th2 cell response which results in elevated antibody production. IL-10 functions by inhibiting pro-inflammatory cytokines made by macrophages and regulatory T cells including IFN-gamma, TNF-alpha, IL-2, and IL-3, IL-4, and GM-CSF. IL-10 is also known to suppress antigen presentation on antigen presenting cells, enhances B cell survival, proliferation, and antibody production. Rat IL-10 ELISA Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates IL-10 in rat serum, plasma, or cell culture medium. The assay will exclusively recognize both natural and recombinant rat IL-10.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0003-48T	GER0003-96T
GER0003-1	Microplate	48T	96T
GER0003-2	Standard	1 vial	2 vials
GER0003-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer

to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum**: Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma**: Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat IL-10 Standard is obtained by redissolving the standard to a concentration of 4000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
   Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated rat IL-10 standard as the highest concentration S7 (2000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

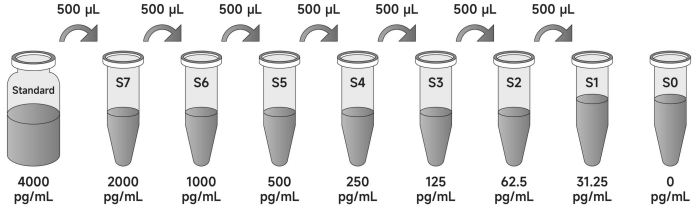
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20℃ to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated rat IL-10 standard as the highest concentration S7 (2000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a

new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.

4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to

remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
2000	2.508	2.624	2.566	2.542
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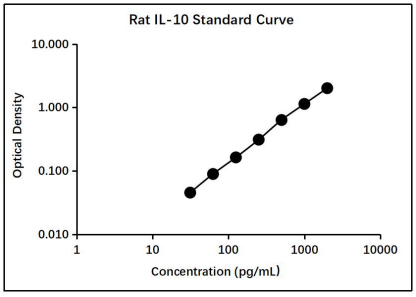
125	0.35	0.337	0.344	0.319
62.50	0.261	0.246	0.254	0.229
31.25	0.181	0.173	0.177	0.153
0	0.076	0.068	0.072	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells.
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[a]: **Sensitivity**

The lower limit of detection of rat IL-10 is 6.08 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<8%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<11%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IL-10 into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 87% to 102%, with a mean recovery of 96%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IL-10 into serum. The linearity range is the ratio of the assay value to the expected value of rat IL-10 in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
89
83-95

1:4
95
91-102

1:8
98
94-101

1:16
102
96-108

Sample values

The kit was applied to 30 normal rat serum samples and all

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat TNF-alpha ELISA Kit

Cat. #: GER0004

Product Information

Product Name	Cat. No.	Spec.
Rat TNF-alpha ELISA Kit	GER0004-48T	48T
	GER0004-96T	96T

Product Description

TNF alpha is a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophage and bind to its receptors, TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. TNF alpha is involved in the regulation of immune cells, cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. Other functions of TNF-alpha include its role in the immune response to bacterial, viral, parasitic and certain fungal infections, as well as its role in the necrosis of specific tumors. TNF alpha causes cytolysis or cytostasis of certain transformed cells, being synergistic with interferon-gamma in its cytotoxicity. This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Rat TNF-alpha ELISA Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates TNF-alpha in rat serum, plasma, cell culture medium. The assay will exclusively recognize both natural and recombinant rat TNF-alpha.

删除[a]: tissue homogenates, cell lysates, or

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0004-48T	GER0004-96T
GER0004-1	Microplate	48T	96T
GER0004-2	Standard	1vial	2vials
GER0004-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum**: Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma**: Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat TNF-alpha Standard is obtained by redissolving the standard to a concentration of 2000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.

    a) Preparation of standard curves for serum/plasma:

    Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated Mouse TNF-alpha standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

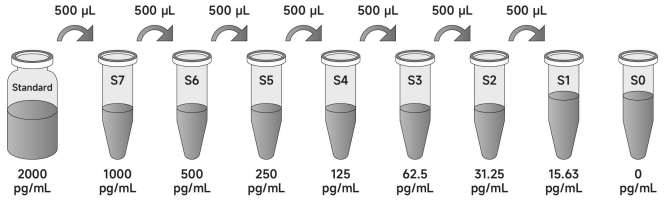
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20℃ to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse TNF-alpha standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminum foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.

4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

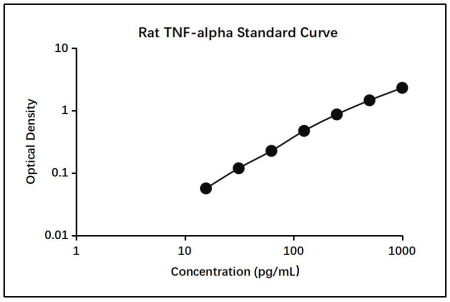
Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
1000	2.540	2.619	2.580	2.515
500	1.574	1.589	1.582	1.517
250	0.960	0.984	0.972	0.907
125	0.562	0.561	0.562	0.497
62.5	0.339	0.354	0.347	0.282
31.25	0.256	0.296	0.276	0.211
15.63	0.192	0.204	0.198	0.133
0	0.065	0.065	0.065	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells.
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wavelength.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12

删除[a]: **Sensitivity**

The lower limit of detection of rat TNF-alpha is 1.82 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV (%) =(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<8%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<10%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat TNF-alpha into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 85% to 103%, with a mean recovery of 93%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat TNF-alpha into serum. The linearity range is the ratio of the assay value to the expected value of rat TNF-alpha in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
88
83-103

1:4
97
82-108

1:8
105
94-112

1:16
109
96-113

Sample values

The kit was applied to 30 normal rat serum samples and all

B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat TNF-alpha ELISA Kit

Cat. #: GER0004

Product Information

Product Name	Cat. No.	Spec.
Rat TNF-alpha ELISA Kit	GER0004-48T	48T
	GER0004-96T	96T

Product Description

TNF alpha is a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophage and bind to its receptors, TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. TNF alpha is involved in the regulation of immune cells, cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. Other functions of TNF-alpha include its role in the immune response to bacterial, viral, parasitic and certain fungal infections, as well as its role in the necrosis of specific tumors. TNF alpha causes cytolysis or cytostasis of certain transformed cells, being synergistic with interferon-gamma in its cytotoxicity. This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Rat TNF-alpha ELISA Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitates TNF-alpha in rat serum, plasma, cell culture medium. The assay will exclusively recognize both natural and recombinant rat TNF-alpha.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0004-48T	GER0004-96T
GER0004-1	Microplate	48T	96T
GER0004-2	Standard	1vial	2vials
GER0004-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum**: Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma**: Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated rat TNF-alpha Standard is obtained by redissolving the standard to a concentration of 2000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.

    a) Preparation of standard curves for serum/plasma:

    Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated Mouse TNF-alpha standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

    b) Preparation of standard curve of cell culture supernatant sample:

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

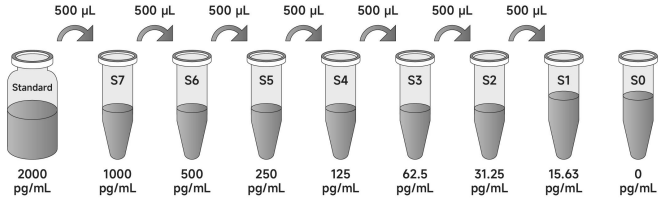
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20℃ to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Mouse TNF-alpha standard as the highest concentration S7 (1000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500 µL of S7 (1000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminum foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 µL of standards of different concentrations to each well, add 100 µL of Diluent A to the blank wells, and 100 µL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 µL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 µL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.

4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

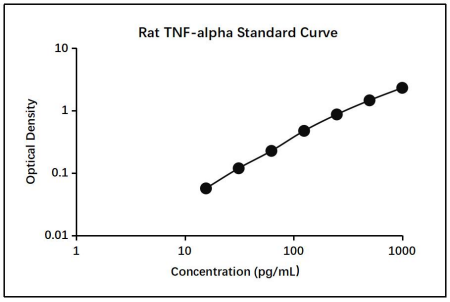
Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
1000	2.540	2.619	2.580	2.515
500	1.574	1.589	1.582	1.517
250	0.960	0.984	0.972	0.907
125	0.562	0.561	0.562	0.497
62.5	0.339	0.354	0.347	0.282
31.25	0.256	0.296	0.276	0.211
15.63	0.192	0.204	0.198	0.133
0	0.065	0.065	0.065	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells.
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wavelength.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										

删除[a]: **Sensitivity**

The lower limit of detection of rat TNF-alpha is 1.82 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV (%) =(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<8%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<10%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat TNF-alpha into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 85% to 103%, with a mean recovery of 93%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat TNF-alpha into serum. The linearity range is the ratio of the assay value to the expected value of rat TNF-alpha in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
88
83-103

1:4
97
82-108

1:8
105
94-112

1:16
109
96-113

Sample values

The kit was applied to 30 normal rat serum samples and all

B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat IFN-gamma ELISA Kit

Cat. #: GER0006

Product Information

Product Name	Cat. No.	Spec.
Rat IFN-gamma ELISA Kit	GER0006-48T	48T
	GER0006-96T	96T

Product Description

IFN-gamma (Interferon gamma, Type II interferon) is a macrophage activation factor, and immune interferon that is produced primarily by T-lymphocytes and natural killer cells in response to antigens, mitogens, Staphylococcus enterotoxin B, phytohemaglutanin and other cytokines. IFN-gamma function includes the following: antiviral activity, tumor antiproliferative activity, induction of class I and II MHC, macrophage activation, and enhanced immunoglobulin secretion by B lymphocytes. IFN-gamma is involved in cytokine regulation and also acts synergistically with other cytokines. The Rat IFN-gamma ELISA Kit uses dual antibody sandwich Enzyme-Linked Immunosorbent assay technology to quantitatively detect IFN-gamma in rat serum, plasma, cell culture supernatant, and other biological fluids. The assay recognizes both natural and recombinant rat IFN-gamma.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0006-48T	GER0006-96T
GER0006-1	Microplate	48T	96T
GER0006-2	Standard	1 vial	2 vials
GER0006-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer

2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma**: Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Rat IFN-gamma Standard is obtained by redissolving the standard to a concentration of 4000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
      Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated Rat IFN-gamma standard as the highest concentration S7 (2000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
      Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Rat IFN-gamma standard as the highest concentration S7 (2000 pg/mL) for the

删除[丽]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.

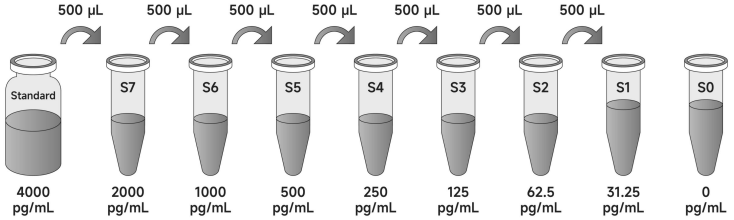
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[丽]: /tissue homogenate/cell lysate

standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 μL each of culture supernatant. Pipette 500 μL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 μL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 μL of standards of different concentrations to each well, add 100 μL of Diluent A to the blank wells, and 100 μL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 μL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 μL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.

5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

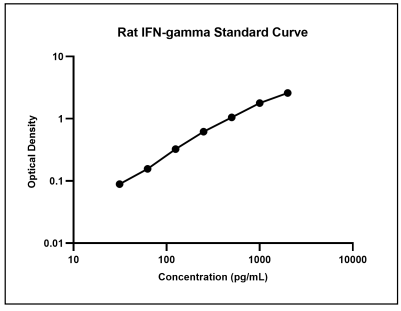
Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
2000	2.646	2.653	2.649	2.597
1000	1.853	1.809	1.831	1.779
500	1.102	1.099	1.101	1.049
250	0.680	0.662	0.671	0.619
125	0.397	0.355	0.376	0.324
62.5	0.215	0.201	0.208	0.156
31.25	0.143	0.138	0.141	0.089
0	0.053	0.051	0.052	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 μL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 μL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 μL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 μL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 μL of stop solution to all wells.
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[丽]: **Sensitivity**

The lower limit of detection of rat IFN-gamma is 19.83 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV (%) =(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<8%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<13%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IFN-gamma into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 89% to 110%, with a mean recovery of 103%.

Linearity of dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IFN-gamma into serum. The linearity range is the ratio of the assay value to the expected value of rat IFN-gamma in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
92
82-109

1:4
102
94-105

1:8
97
90-112

1:16
103
90-112

Sample values

The kit was applied to 30 normal rat serum samples and

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat IFN-gamma ELISA Kit

Cat. #: GER0006

Product Information

Product Name	Cat. No.	Spec.
Rat IFN-gamma ELISA Kit	GER0006-48T	48T
	GER0006-96T	96T

Product Description

IFN-gamma (Interferon gamma, Type II interferon) is a macrophage activation factor, and immune interferon that is produced primarily by T-lymphocytes and natural killer cells in response to antigens, mitogens, Staphylococcus enterotoxin B, phytohemaglutanin and other cytokines. IFN-gamma function includes the following: antiviral activity, tumor antiproliferative activity, induction of class I and II MHC, macrophage activation, and enhanced immunoglobulin secretion by B lymphocytes. IFN-gamma is involved in cytokine regulation and also acts synergistically with other cytokines. The Rat IFN-gamma ELISA Kit uses dual antibody sandwich Enzyme-Linked Immunosorbent assay technology to quantitatively detect IFN-gamma in rat serum, plasma, cell culture supernatant, and other biological fluids. The assay recognizes both natural and recombinant rat IFN-gamma.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0006-48T	GER0006-96T
GER0006-1	Microplate	48T	96T
GER0006-2	Standard	1 vial	2 vials
GER0006-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).

2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma**: Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Rat IFN-gamma Standard is obtained by redissolving the standard to a concentration of 4000 pg/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.
   a) Preparation of standard curves for serum/plasma:
      Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated Rat IFN-gamma standard as the highest concentration S7 (2000 pg/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
      Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Rat IFN-gamma standard as the highest concentration S7 (2000 pg/mL) for the

删除[丽]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.

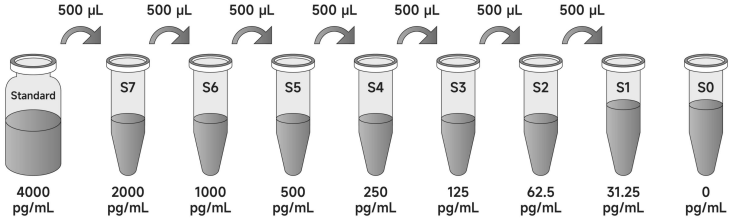
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[丽]: /tissue homogenate/cell lysate

standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 μL each of culture supernatant. Pipette 500 μL of S7 (2000 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 μL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 μL of standards of different concentrations to each well, add 100 μL of Diluent A to the blank wells, and 100 μL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.

   Note: Refer to the relevant literature to determine the approximate concentration of the protein to be tested in the sample. If the concentration is greater or less than the maximum or minimum standard concentration of the kit, dilute or concentrate it appropriately before testing.
2. **Plate washing:** Automatic or manual plate washer with 300 μL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 μL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.

5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.
6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

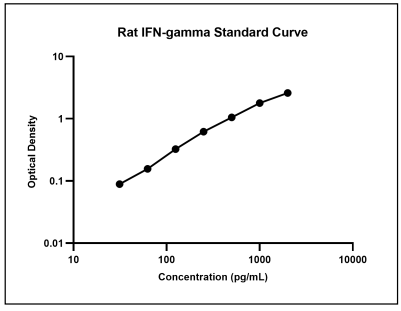
Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.
   c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.
   d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.
2. **Typical data**
   Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

pg/mL	OD		Average	Corrected
2000	2.646	2.653	2.649	2.597
1000	1.853	1.809	1.831	1.779
500	1.102	1.099	1.101	1.049
250	0.680	0.662	0.671	0.619
125	0.397	0.355	0.376	0.324
62.5	0.215	0.201	0.208	0.156
31.25	0.143	0.138	0.141	0.089
0	0.053	0.051	0.052	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells.
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

删除[丽]: **Sensitivity**

The lower limit of detection of rat IFN-gamma is 19.83 pg/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV (%) =(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<8%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<13%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of rat IFN-gamma into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 89% to 110%, with a mean recovery of 103%.

Linearity of dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of rat IFN-gamma into serum. The linearity range is the ratio of the assay value to the expected value of rat IFN-gamma in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
92
82-109

1:4
102
94-105

1:8
97
90-112

1:16
103
90-112

Sample values

The kit was applied to 30 normal rat serum samples and

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat C-Reactive Protein (CRP) ELISA Kit

Cat. #: GER0007

Product Information

Product Name	Cat. No.	Spec.
Rat C-Reactive Protein (CRP) ELISA Kit	GER0007-48T	48T
	GER0007-96T	96T

Product Introduction

CRP (C-reactive protein) is a major cyclic, pentameric acute phase protein compound consisting of five identical, noncovalently bound, nonglycosylated subunits. CRP is produced by the liver, and its plasma levels rise dramatically during inflammatory processes occurring in the body. CRP plays an important role in innate immunity. According to current models, proinflammatory cytokines produced at localized sites of injury or inflammation such as IL-6, is transported by the bloodstream to the liver where they modulate the expression of C-reactive protein and other acute phase reactants. Rat C-Reactive Protein(CRP) ELISA Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitative measurement of Rat CRP in serum, plasma, cell culture supernatants or other relevant fluids, and for the simultaneous detection of natural and recombinant Rat CRP.

删除[a]: tissue homogenates, cell lysates,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0007-48T	GER0007-96T
GER0007-1	Microplate	48T	96T
GER0007-2	Standard	1 vial	2 vials
GER0007-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450 nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).

2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum**: Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma**: Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Rat CRP Standard is obtained by redissolving the standard to a concentration of 20 ng/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.

a) Preparation of standard curves for serum/plasma:
Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated Rat CRP standard as the highest concentration S7 (10 ng/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (10 ng/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

b) Preparation of standard curve of cell culture supernatant sample:
Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Rat CRP standard as the highest concentration S7 (10 ng/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

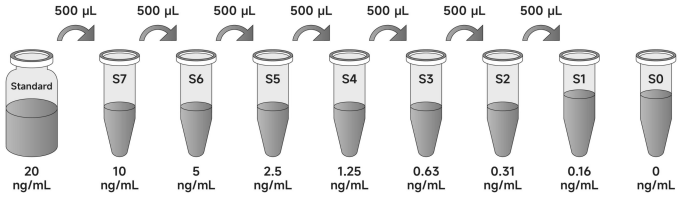
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

μL of S7 (10 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 μL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 μL of standards of different concentrations to each well, add 100 μL of Diluent A to the blank wells, and 100 μL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.
   Note: The recommended dilution ratio for serum samples is 1:2000.
2. **Plate washing:** Automatic or manual plate washer with 300 μL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 μL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.

6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.

b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

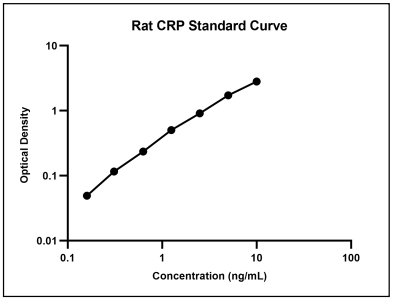
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

ng/mL	OD		Average	Corrected
10	2.879	2.812	2.846	2.815
5	1.777	1.723	1.750	1.719
2.5	0.974	0.902	0.938	0.907
1.25	0.549	0.521	0.535	0.504
0.63	0.273	0.258	0.266	0.235
0.31	0.151	0.143	0.147	0.116
0.16	0.067	0.092	0.080	0.049
0	0.030	0.032	0.031	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells.
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										

删除[a]: **Sensitivity**

The lower limit of detection of Rat CRP is 0.06 ng/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean) ×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<6%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<8%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of Rat CRP into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 88% to 106%, with a mean recovery of 98%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of Rat CRP into serum. The linearity range is the ratio of the assay value to the expected value of Rat CRP in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
96
93-98

1:4
94
88-105

1:8
105
98-110

1:16
102
99-110

Sample values

The kit was applied to 30 healthy rat serum/plasma

C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Rat C-Reactive Protein (CRP) ELISA Kit

Cat. #: GER0007

Product Information

Product Name	Cat. No.	Spec.
Rat C-Reactive Protein (CRP) ELISA Kit	GER0007-48T	48T
	GER0007-96T	96T

Product Introduction

CRP (C-reactive protein) is a major cyclic, pentameric acute phase protein compound consisting of five identical, noncovalently bound, nonglycosylated subunits. CRP is produced by the liver, and its plasma levels rise dramatically during inflammatory processes occurring in the body. CRP plays an important role in innate immunity. According to current models, proinflammatory cytokines produced at localized sites of injury or inflammation such as IL-6, is transported by the bloodstream to the liver where they modulate the expression of C-reactive protein and other acute phase reactants. Rat C-Reactive Protein(CRP) ELISA Kit is a dual antibody sandwich enzyme-linked immunosorbent assay for the in vitro quantitative measurement of Rat CRP in serum, plasma, cell culture supernatants or other relevant fluids, and for the simultaneous detection of natural and recombinant Rat CRP.

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GER0007-48T	GER0007-96T
GER0007-1	Microplate	48T	96T
GER0007-2	Standard	1 vial	2 vials
GER0007-3	Detection Antibody	60 μL	120 μL
G0023	SA-HRP	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450 nm. The reference wavelength is 630 nm (Refer to the instruction manual supplied with the instrument to pre-warm).

2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection And Storage Instructions

1. **Serum**: Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma**: Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant:** Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate(25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Standards: Add Diluent A to the standard in the labeled volume and gently vortex and shake to ensure complete mixing. The concentrated Rat CRP Standard is obtained by redissolving the standard to a concentration of 20 ng/mL. Allow to stand for 10 minutes after re-dissolution and mix well before dilution.

a) Preparation of standard curves for serum/plasma:
Mix the reconstituted standards well, add 500 µL of Dilution A to 500 µL of concentrated Rat CRP standard as the highest concentration S7 (10 ng/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of Dilution A. Pipette 500 µL of S7 (10 ng/mL) standard into the first tube S6, and mix gently. Pipette 500 µL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is diluent A.

b) Preparation of standard curve of cell culture supernatant sample:
Mix the reconstituted standards well, add 500 µL of culture supernatant to 500 µL of concentrated Rat CRP standard as the highest concentration S7 (10 ng/mL) for the standard curve. Take six 1.5 mL centrifuge tubes (S1-S6) arrange in sequence and add 500 µL each of culture supernatant. Pipette 500

删除[a]: **Tissue homogenates**: The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

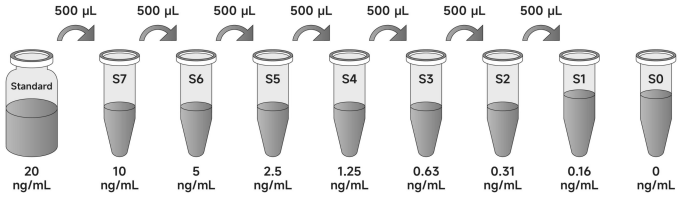
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 µL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 µL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

μL of S7 (10 pg/mL) standard into the first tube S6, and mix gently. Pipette 500 μL from S6 into the second centrifuge tube S5 and mix with gentle blowing. And so on for multiplicative dilution of standards. S0 is culture supernatant.



4. 1x detection antibody: Centrifuge briefly and make a 1:100 dilution of detection antibody with Diluent B to a 1x detection antibody working solution prior to use.
5. 1×SA-HRP: Centrifuge briefly and make a 1:100 dilution of SA-HRP with Diluent B to a 1x SA-HRP working solution prior to use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standards and samples with Diluent A. Set up 7 standard wells (S1-S7), add 100 μL of standards of different concentrations to each well, add 100 μL of Diluent A to the blank wells, and 100 μL of the sample to be tested to the remaining wells, cover the plate with a plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and can be mixed well), incubate and shake at room temperature for 2 hours.
   Note: The recommended dilution ratio for serum samples is 1:2000.
2. **Plate washing:** Automatic or manual plate washer with 300 μL of wash buffer per well, the interval between injection and aspiration is 15-30 seconds and wash 5 times. After the last washing, the plate is inverted and patted dry on absorbent paper with appropriate force and the liquid in the wells is discarded.
3. **Add Detection Antibody:** Dilute the detection antibody to working concentration with Diluent B, add 1x detection antibody working solution 100 μL per well (prepared before use), seal the plate with a new sealing film, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and is well mixed) and incubate for 1 hour at room temperature with shaking.
4. **Plate washing:** Repeat step 2.
5. **Add SA-HRP:** Dilute SA-HRP to working concentration with Diluent B. Add 100 μL of 1x SA-HRP working solution (prepared before use) to each well, cover the plate with a new plate sealer, shake at 100-300 rpm (to ensure that the solution does not spill out of each well and that it is well mixed) and incubate for 30 minutes at room temperature.

6. **Plate washing:** Repeat step 2.
7. **Add TMB Substrate:** Add 90 μL of TMB substrate to each well, replace with a new plate sealer and develop the colour at room temperature protected from light (the reaction time should be controlled for 10-30 minutes. Terminate when the first 3-4 wells of the standard have a clear gradient of blue and the last 3-4 wells have no clear gradient).
8. **Add Stop Solution:** The reaction is terminated by adding 50 μL of stop solution to each well, at which point the blue colour immediately turns yellow. The stop solution should be added in the same order as the substrate solution as far as possible. In case of uneven colour, shake the plate gently to mix the solution well.
9. **Reading:** After ensuring that the bottom of the plate is free of water droplets and the wells are free of air bubbles, reading absorbance at 450 nm within 10 minutes. It is recommended to use dual wavelengths. Use 450 nm as detection wavelength, 630 nm as reference and calibration wavelength. Measure at 450 nm only will reduce accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results Calculation**
   a) To obtain more accurate results, it is recommended to calculate the average absorbance values for each set of duplicate standards and samples.

b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

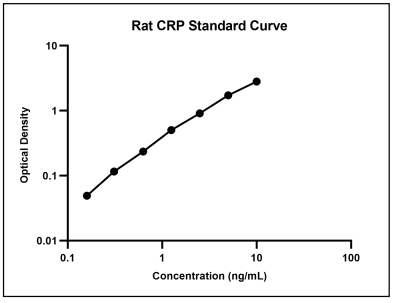
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

ng/mL	OD		Average	Corrected
10	2.879	2.812	2.846	2.815
5	1.777	1.723	1.750	1.719
2.5	0.974	0.902	0.938	0.907
1.25	0.549	0.521	0.535	0.504
0.63	0.273	0.258	0.266	0.235
0.31	0.151	0.143	0.147	0.116
0.16	0.067	0.092	0.080	0.049
0	0.030	0.032	0.031	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add 100 µL of sample (standard or sample) to all wells and shake for 2 hours at room temperature.
3. Wash 5 times and pat dry, add 100 µL of detection antibody working solution to all wells and shake for 1 hour at room temperature.
4. Wash 5 times and pat dry, add 100 µL of SA-HRP working solution to all wells and shake for 30 minutes at room temperature.
5. Wash 5 times and pat dry, add 90 µL of TMB substrate to all wells and incubate for 10-30 minutes at room temperature in the dark.
6. Add 50 µL of stop solution to all wells.
7. Read absorbance at 450 nm within 10 minutes, optionally 630 nm as the reference wave length.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										

删除[a]: **Sensitivity**

The lower limit of detection of Rat CRP is 0.06 ng/mL (mean of 3 independent assays).

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean) ×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<6%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<8%.

Spike recovery

The spike recovery was evaluated by spiking 5 levels of Rat CRP into serum. repeat the measurement and calculate the mean. The unspiked serum was used as blank in these experiments and the recovery was calculated (recovery is the ratio of the assay value to the expected value). The recoveries range from 88% to 106%, with a mean recovery of 98%.

Linearity of Dilution

Linearity of dilution was determined by serially diluting sample in the kinetic range of the standard curve after spiking 5 levels of Rat CRP into serum. The linearity range is the ratio of the assay value to the expected value of Rat CRP in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
96
93-98

1:4
94
88-105

1:8
105
98-110

1:16
102
99-110

Sample values

The kit was applied to 30 healthy rat serum/plasma

C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

删除[a]: V1

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Servicebio® Rat IgG ELISA Kit

Cat. #：GER0013

Product Information

Product Name	Cat. No.	Spec.
Rat IgG ELISA Kit	GER0013-48T	48T
	GER0013-96T	96T

Product Description

ImmunoglobulinG (IgG) is the most important form of immunoglobulin in serum, accounting for about 75% of the total content of immunoglobuling in serum, and is the most important antibody component in serum and extracellular fluid, mainly involved in the identification and elimination of pathogens and toxic substances, and has antiviral, antibacterial and immunomodulatory functions.IgG is a four-stranded monomer, consisting of two light and two heavy chains.Structurally, it can be divided into variable region and constant region, wherein variable region has antigen recognition site.IgG is also the only antibody that crosses the placenta during pregnancy to protect the fetus and plays an important role in fighting infection in the newborn.RatIgGELISAKit can quantitatively detect IgG in rat serum, plasma, cell culture supernatant or other related liquids in vitro through double antibody sandwich ELISA technique, and can detect both natural and recombinant rat IgG.

删除[a]: tissue homogenate, cell lysate,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 12 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GEH0014-48T	GEH0014-96T
GER0013-1	Microplate	48T	96T
GER0013-2	Standard	1 vial	2 vials
G0030	Enzyme-labeled Antibody	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL
G0025	Diluent B	30 mL	30 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1 pc	1 pc

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm(Refer to the instruction manual supplied with the instrument to pre-warm).

2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.
4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate (25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Preparation of standard product: Add diluent A to the standard product according to the volume marked on the label, and gently swirl to ensure full mixing.The standard product concentration after redissolution is 100ng/mL, that is, the concentrated Rat IgG standard product. Let stand for 10 minutes after remelting and mix well before diluting.
   a) Preparation of standard curves for serum/plasma:
      After fully mixing the redissolved standard product, 500μL concentrated rat IgG standard product was taken and added with 500μL diluent A as the highest concentration S7 (50ng/mL) on the standard curve.Six 1.5mL centrifuge tubes (S1-S6) were arranged successively, and 500μL diluent A was added to each tube.Draw 500μLS7 (50ng/mL) standard into the first centrifuge tube S6 and gently blow and mix.Draw 500μL from S6 into the second centrifuge tube S5 and gently blow and mix.And so on to double dilution of standard products.S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
      After fully mixing the redissolved standard product, 500μL concentrated rat IgG standard product was taken and added into 500μL cell medium as the highest concentration S7 (50ng/mL) of the standard curve.Six 1.5mL centrifuge tubes (S1-S6) were sequentially arranged and 500μL cell medium was added to each.Draw 500μLS7 (50ng/mL) standard into the first centrifuge tube S6

删除[a]: **Tissue homogenates:** The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

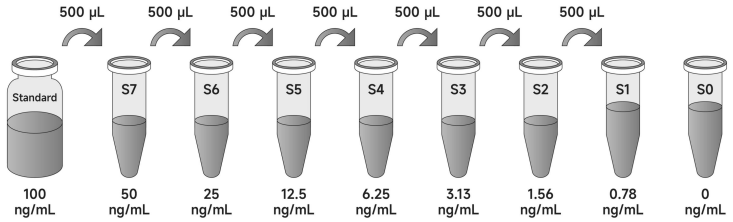
Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 μL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 μL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate

and gently blow and mix.Draw 500μL from S6 into the second centrifuge tube S5 and gently blow and mix.And so on to double dilution of standard products.S0 is the cell medium.



4. 1× enzymic antibody: The enzymic antibody is briefly centrifuged and diluted to the working concentration by 1:100 times with diluent B.1× enzyme-labeled antibody working liquid was mixed and prepared before clinical use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Standard holes, sample holes to be measured and blank holes are set up respectively.Dilute the standard product and sample with diluent A, set standard holes 7 holes (S1-S7), add 100μL standard product of different concentration to each hole successively, add 100μL diluent A to each hole, add 100μL sample to each hole, seal the plate membrane, and oscillate at 100-300rpm (ensure that the solution does not spill out of each hole and can be fully mixed).Incubate at room temperature for 2 hours;

   Note: Please refer to relevant literature to determine the approximate concentration of the protein to be detected in the sample. If the concentration is greater than or less than the maximum or minimum standard concentration of this kit, please carry out appropriate dilution or concentration before testing.
2. **Wash plate:** Automatic washing plate or manual washing plate, each hole of the washing liquid is 300μL, injection and suction interval of 15-30 seconds.Wash the board five times.After the last plate washing is completed, the enzyme label plate is upside-down on the absorbent paper and patted dry properly, and the liquid in the hole is discarded;
3. **Add the enzyme-labeled antibody:** Dilute the enzyme-labeled antibody to the working concentration with diluant B, add the enzyme-labeled antibody working solution (prepared before use) 100μL in each well, replace the sealing plate with a new sealing plate, shake at 100-300rpm (ensure that the solution does not spill in each well and can be fully mixed), and incubate at room temperature for 30 minutes;
4. **Plate washing:** Repeat step 2.

5. **Add TMB Substrate:** Add TMB substrate solution 90μL to each hole, replace the new sealing plate film, and color rendering at room temperature (the reaction time should be controlled within 10-30 minutes, not more than 30 minutes.When the first 3-4 holes of the standard product have obvious gradient blue, the gradient of the last 3-4 holes is not obvious, it can be terminated);
6. **Add Stop Solution:** Add termination solution 50μL to each well to terminate the reaction, and the blue immediately turns to yellow.The addition sequence of the termination solution should be as close as possible to the addition sequence of the substrate solution.If the color is uneven, please gently shake the label plate to make the solution evenly mixed;
7. **Reading:** .After ensuring that there are no water droplets at the bottom of the enzyme label plate and no bubbles in the hole, the detection wavelength of 450nm is used to read the value within 10 minutes.It is recommended to use dual wavelengths, namely the detection wavelength of 450nm, the reference wavelength, or the correction wavelength of 630nm to read the value at the same time. Using only 450nm will reduce the accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.

b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

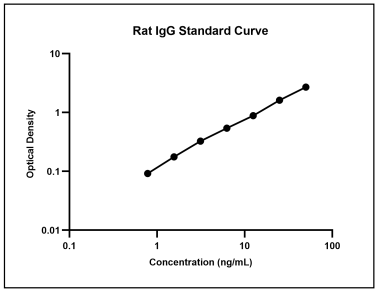
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

ng/mL	OD		Average	Corrected
50	2.725	2.743	2.734	2.706
25	1.598	1.690	1.644	1.616
12.5	0.915	0.909	0.912	0.884
6.25	0.566	0.574	0.570	0.542
3.13	0.354	0.356	0.355	0.327
1.56	0.204	0.203	0.204	0.176
0.78	0.124	0.116	0.120	0.092
0	0.028	0.027	0.028	



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add sample (standard or sample) 100μL/ well, oscillate at room temperature for 2 hours;
3. After washing for 5 times, pat dry, add enzyme-labeled antibody working solution 100μL/ well, and shake at room temperature for 30 minutes;
4. Wash for 5 times and pat dry, add TMB substrate 90μL/ well, incubate at room temperature for 10-30 minutes away from light;
5. Add termination solution 50μL/ well;
6. The OD value is detected at 450 nm wavelength within 10 minutes, with a reference wavelength of 630 nm.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										

删除[a]: **Sensitivity**

The lowest detectable concentration of rat IgG was 0.075ng/mL(the mean of 3 independent experiments).This value is the average OD value measured by 20 blank holes plus the concentration value corresponding to twice SD.

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<9%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<15%.

Spike recovery

A certain amount of rat IgG (labeled sample) was added to 5 samples of fixed rat serum, repeated determination was made and the mean value was calculated. The serum without increasing rat IgG was used as the background, and the recovery rate was calculated (the ratio of measured value to theoretical value).Recovery rates range from 90% to 99%, with an average recovery rate of 93%.

Linearity of dilution

A certain amount of rat IgG (labeled sample) was added to 5 samples of fixed rat serum, and a series of dilution was performed within the kinetic range of the standard curve to evaluate the linearity of the detection.The linear range is the ratio of the measured and theoretical IgG content of rats in the diluted sample.

Dilution
Mean(%)
Range(%)

1:2
98
89-108

1:4
103
94-109

1:8
96
90-109

1:16
90
85-105

Sample values

Serum samples from 30 healthy rats were analyzed with this kit, and all samples were positive.

C	S5	S5										
D	S4	S4										
E	S3	S3										
F	S2	S2										
G	S1	S1										
H	S0	S0										

Servicebio® Cortisol ELISA Kit（Common Species）

Cat. #：GE0002

Product Information

Product Name	Cat. No.	Spec.
Cortisol ELISA Kit	GE0002-48T	48T
	GE0002-96T	96T

Product Description

Cortisol is a steroid hormone secreted by the adrenal cortex, also known as hydrocortisone.Cortisol plays a very important role in maintaining the stability of physiological functions and regulating the metabolism of protein, fat and sugar, and also has a strong anti-inflammatory, anti-shock and anti-allergic effect, with obvious circadian rhythm.Cortisol is needed by the body to maintain normal physiological functions during stress, so it is also known as the stress hormone.Cortisol ELISA Kit quantitatively assesses Cortisol in vitro in serum, plasma, cell culture supernatant, or other related liquids through competitive ELISA. Cortisolelisakit can detect both natural and synthetic Cortisol.

删除[a]: tissue homogenate, cell lysate,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 6 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GEH0014-48T	GEH0014-96T
GE0002-1	Microplate	48T	96T
GE0002-2	Standard	1 vial	2 vials
GE0002-3	Detection Antibody	30 μL	60 μL
G0030	Enzyme-labeled Antibody	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL * 2
G0025	Diluent B	12 mL	12 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1pc	1pc

删除[丽]: Precoated Enzyme

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删除[a]: 40

删除[a]: 80

Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm(Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.

4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

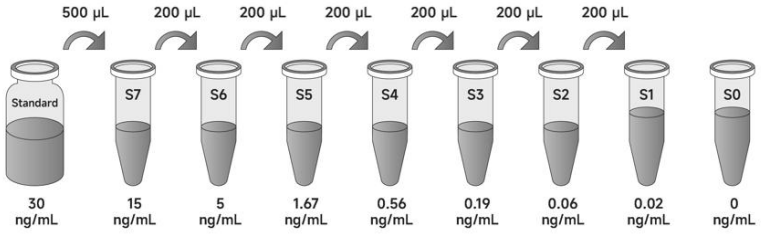
1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate (25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Preparation of standard product: Add diluent A to the standard product according to the volume marked on the label, and gently swirl to ensure full mixing.The standard product concentration after redissolution is 30ng/mL, which is the concentrated Cortisol standard product.Let stand for 10 minutes after remelting and mix well before diluting.
   a) Preparation of standard curves for serum/plasma
   Fully mixed the redissolved standard product, 500μL concentrated Cortisol standard product was added to 500μL diluent A as the highest concentration S7 (15ng/mL) of the standard curve.Six 1.5mL centrifuge tubes (S1-S6) were arranged successively and 400μL diluent A was added to each tube.Draw 200μLS7 (15ng/mL) standard into the first centrifuge tube S6, gently blow and mix.Draw 200μL from S6 into the second centrifuge tube S5 and gently blow and mix.And so on to the standard product 3 times the ratio dilution.S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
   Fully mixed the redissolved standard product, 500μL concentrated standard product was added



删除[a]: **Tissue homogenates:** The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 μL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 μL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate:

to 500μL cell medium as the highest concentration S7 (15ng/mL) on the standard curve.Six 1.5mL centrifuge tubes (S1-S6) were sequentially arranged and 400μL cell medium was added to each.Draw 200μLS7 (15ng/mL) standard into the first centrifuge tube S6, gently blow and mix.Draw 200μL from S6 into the second centrifuge tube S5 and gently blow and mix.And so on to the standard product 3 times the ratio dilution.S0 is the cell medium.

4. 1× antibody preparation: The antibody is briefly centrifuged and diluted to the working concentration by 1:100 times with diluent A. 1× antibody working liquid is mixed and prepared before clinical use.
5. Preparation of 1× enzymic antibody: The enzymic antibody is briefly centrifuged and diluted to the working concentration by 1:100 times with diluent B. 1× enzyme-labeled antibody working liquid is mixed and prepared before clinical use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standard product and sample with diluent A, set standard holes 7 holes (S1-S7), add 100μL standard product of different concentration to each hole successively, add 100μL diluent A to each blank hole, and add 100 μL sample to each remaining hole.
2. **Add antibody:** Dilute antibody to working concentration with diluent A, add antibody 50μL per well.Sealing plate film sealing plate, 100-300rpm oscillation (ensure that each hole solution does not spill and can be fully mixed), temperature oscillation incubation for 1 hour.

   Note: 1. Complete steps 1 and 2 as soon as possible with an interval of no more than 10 minutes. The sequence of steps cannot be changed.

   2. Please refer to relevant literature to determine the approximate concentration of the protein to be detected in the sample. If the concentration is greater than or less than the maximum or minimum standard concentration of this kit, please carry out appropriate dilution or concentration before testing.
3. **Wash plate:** automatic washing plate or manual washing plate, each hole of the washing liquid is 300μL, injection and suction interval of 15-30 seconds.Wash the board five times.After the last plate washing is completed, the enzyme label plate is upside-down on the absorbent paper and patted dry properly, and the liquid in the hole is discarded;
4. **Add the enzyme-labeled antibody:** Dilute the enzyme-labeled antibody to the working concentration with diluant B, add the enzyme-labeled antibody working solution (prepared before use) 100μL in each well, replace the sealing plate with a new sealing plate, shake at 100-300rpm (ensure that the solution does not spill in each well and can be fully mixed), and incubate at room temperature for 30 minutes;
5. **Plate washing:** Repeat step 3.
6. **Add TMB Substrate:** Add TMB substrate solution 90μL to each hole, replace the new sealing plate film, and color rendering at room temperature (the reaction time should be controlled within 10-30

minutes, not more than 30 minutes.When the first 3-4 holes of the standard product have obvious gradient blue, the gradient of the last 3-4 holes is not obvious, it can be terminated);

7. **Add Stop Solution:** Add termination solution 50μL to each well to terminate the reaction, and the blue immediately turns to yellow.The addition sequence of the termination solution should be as close as possible to the addition sequence of the substrate solution.If the color is uneven, please gently shake the label plate to make the solution evenly mixed;
8. **Reading:** .After ensuring that there are no water droplets at the bottom of the enzyme label plate and no bubbles in the hole, the detection wavelength of 450nm is used to read the value within 10 minutes.It is recommended to use dual wavelengths, namely the detection wavelength of 450nm, the reference wavelength, or the correction wavelength of 630nm to read the value at the same time. Using only 450nm will reduce the accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation

coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

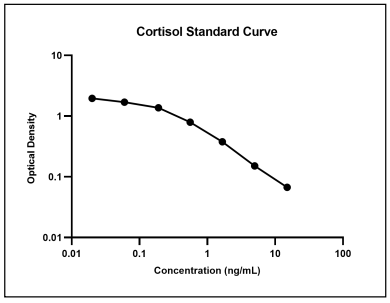
c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

ng/mL	OD		Average
15	0.070	0.064	0.067
5	0.159	0.143	0.151
1.67	0.383	0.371	0.377
0.56	0.789	0.795	0.792
0.19	1.364	1.368	1.366
0.06	1.709	1.681	1.695
0.02	1.923	1.986	1.955
0	2.219	2.203	2.211



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add sample (standard product or sample) 100μL/ well, then add antibody working solution 50μL/ well, and add sample continuously within 10 minutes.After mixing, shake at room temperature for 1 hour;
3. After washing for 5 times, pat dry, add enzyme-labeled antibody working solution 100μL/ well, and shake at room temperature for 30 minutes;
4. Wash for 5 times and pat dry, add TMB substrate 90μL/ well, incubate at room temperature for 10-30 minutes away from light;
5. Add termination solution 50μL/ well;
6. The OD value is detected at 450 nm wavelength within 10 minutes, with a reference wavelength of 630 nm.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										

删除[a]: **Sensitivity**

The lowest detectable concentration of Cortisol is 8.6pg/mL(the average of three independent experiments).This value is the average OD value measured by 20 blank holes plus the concentration value corresponding to twice SD

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<9%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<15%.

Spike recovery

A certain amount of Cortisol (labeled samples) was added to 5 sets of normal human serum, and the mean value was measured repeatedly, and the recovery rate was calculated (the ratio of measured value to theoretical value) for the serum without Cortisol.Recovery rates range from 80% to 107%, with an average recovery rate of 92%.

Linearity of dilution

A certain amount of Cortisol (labeled samples) was added to five sets of human serum and diluted in a series within the kinetic range of a standard curve to evaluate the linearity of the test.The linear range is the ratio of Cortisol content measured to theoretical value in a diluted sample.

Dilution
Mean(%)
Range(%)

1:2
93
85-108

1:4
95
89-101

1:8
108
94-118

1:16
105
99-115

Sample values

The serum/plasma samples of 30 healthy volunteers were analyzed with this kit. The drug use history of the volunteers was unknown, and all samples were positive.

F	S2	S2										
G	S1	S1										
H	S0	S0										

删除[a]: V1

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Servicebio® Cortisol ELISA Kit（Common Species）

Cat. #：GE0002

Product Information

Product Name	Cat. No.	Spec.
Cortisol ELISA Kit	GE0002-48T	48T
	GE0002-96T	96T

Product Description

Cortisol is a steroid hormone secreted by the adrenal cortex, also known as hydrocortisone.Cortisol plays a very important role in maintaining the stability of physiological functions and regulating the metabolism of protein, fat and sugar, and also has a strong anti-inflammatory, anti-shock and anti-allergic effect, with obvious circadian rhythm.Cortisol is needed by the body to maintain normal physiological functions during stress, so it is also known as the stress hormone.Cortisol ELISA Kit quantitatively assesses Cortisol in vitro in serum, plasma, cell culture supernatant, or other related liquids through competitive ELISA. Cortisolelisakit can detect both natural and synthetic Cortisol.

删除[a]: tissue homogenate, cell lysate,

Storage and Shipping Conditions

Ship with wet ice; Store at 4℃, valid for 6 months; Use within 4 weeks once opened.

Product Components

Component Number	Component	GEH0014-48T	GEH0014-96T
GE0002-1	Microplate	48T	96T
GE0002-2	Standard	1 vial	2 vials
GE0002-3	Detection Antibody	30 μL	60 μL
G0030	Enzyme-labeled Antibody	60 μL	120 μL
G0024	Diluent A	30 mL	30 mL * 2
G0025	Diluent B	12 mL	12 mL
G0026	TMB Substrate	6 mL	11 mL
G0027	Stop Solution	6 mL	6 mL
G0028	25x Wash Buffer	30 mL	30 mL
G6077	Plate Sealers	4 pcs	4 pcs
Manual		1pc	1pc

删除[丽]: Precoated Enzyme

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删除[丽]: P

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删除[a]: 40

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Additional Materials Required

1. Microtiter plate reader capable of measurement at 450nm. The reference wavelength is 630 nm(Refer to the instruction manual supplied with the instrument to pre-warm).
2. Single or multichannel pipettes and tips, loading slots, centrifuge tubes.
3. Deionized or distilled water.

4. Dry filter or absorbent paper.
5. Vortex mixer, microplate oscillator.

Sample Collection and Storage Instructions

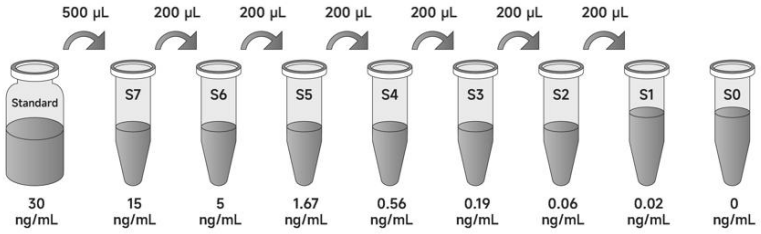
1. **Serum:** Collect samples to clot for 30 minutes at room temperature before centrifugation at 1000 x g for 15 minutes at 2-8°C. Collect the supernatant to carry out the assay, make aliquots and store at -20℃ to avoid repeated freez-thaw cycles.
2. **Plasma:** Collect plasma with EDTA, sodium citrate or heparin as anticoagulants, and centrifuge at 1000 x g for 15 minutes at 2-8°C within 30 minutes of collection, aspirate supernatant for testing, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.
3. **Cell culture supernatant**: Centrifuge at 300 x g for 10 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

Note

1. Centrifuge to remove precipitate if sample is cloudy, not suitable for grossly hemolyzed and lipemic samples.
2. All the above samples should be sealed and stored for no more than 1 week at 4°C, 1 month at -20°C and 2 months at -80°C.
3. Gradually equilibrate frozen samples to room temperature before beginning assay.

Reagent Preparation

1. Remove the kit and samples from the refrigerator 20 minutes in advance and equilibrate to room temperature. Remove the plates and reagents as required for the experiment and store the remaining reagents at 4℃.
2. 1x Wash Buffer: Pour 30 mL of the Wash Buffer Concentrate (25x) into a 1000 mL graduated cylinder. Bring to final volume of 750 mL with distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-25℃.
3. Preparation of standard product: Add diluent A to the standard product according to the volume marked on the label, and gently swirl to ensure full mixing.The standard product concentration after redissolution is 30ng/mL, which is the concentrated Cortisol standard product.Let stand for 10 minutes after remelting and mix well before diluting.
   a) Preparation of standard curves for serum/plasma
   Fully mixed the redissolved standard product, 500μL concentrated Cortisol standard product was added to 500μL diluent A as the highest concentration S7 (15ng/mL) of the standard curve.Six 1.5mL centrifuge tubes (S1-S6) were arranged successively and 400μL diluent A was added to each tube.Draw 200μLS7 (15ng/mL) standard into the first centrifuge tube S6, gently blow and mix.Draw 200μL from S6 into the second centrifuge tube S5 and gently blow and mix.And so on to the standard product 3 times the ratio dilution.S0 is diluent A.
   b) Preparation of standard curve of cell culture supernatant sample:
   Fully mixed the redissolved standard product, 500μL concentrated standard product was added



删除[a]: **Tissue homogenates:** The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice. The homogenate was homogenised by adding 10 times the weight of the tissue in PBS and sonicated until clarified. Centrifuge at 12,000 x g for 5 minutes, discard the precipitate and aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

Cell lysates

For adherent cells: Wash the cells with PBS 2-3 times and aspirate the residual liquid thoroughly. Aspirate cell lysate at a ratio of 250 μL lysate per well of cells in 6-well plates and flasks, and shake repeatedly to bring the lysate into full contact with the cells for 3-5 minutes. Scrape off the cells with a cell spatula and collect in a centrifuge tube. Centrifuge at 12,000 x g for 5 minutes, aspirate supernatant to test, make aliquots and store at -20°C to avoid repeated freez-thaw cycles.

For suspension cells: Collect cells by centrifugation and mix with cell lysate at a ratio of 250 μL of lysate per well of a 6-well plate and shaken. Ice bath for 30 minutes, repeatedly pipetting every 10 minutes to ensure complete cell lysis. Centrifuge at 12,000 x g for 5 minutes and aspirate the supernatant to assay, make aliquots and store the supernatant at -20°C to avoid repeated freez-thaw cycles.

删除[a]: /tissue homogenate/cell lysate:

to 500μL cell medium as the highest concentration S7 (15ng/mL) on the standard curve.Six 1.5mL centrifuge tubes (S1-S6) were sequentially arranged and 400μL cell medium was added to each.Draw 200μLS7 (15ng/mL) standard into the first centrifuge tube S6, gently blow and mix.Draw 200μL from S6 into the second centrifuge tube S5 and gently blow and mix.And so on to the standard product 3 times the ratio dilution.S0 is the cell medium.

4. 1× antibody preparation: The antibody is briefly centrifuged and diluted to the working concentration by 1:100 times with diluent A. 1× antibody working liquid is mixed and prepared before clinical use.
5. Preparation of 1× enzymic antibody: The enzymic antibody is briefly centrifuged and diluted to the working concentration by 1:100 times with diluent B. 1× enzyme-labeled antibody working liquid is mixed and prepared before clinical use.

Note

1. Unopened kits: Store entirely at 4°C and valid for 12 months.
2. Opened kits: Lyophilised standards should not be reused after dissolution and remaining reagents should be stored promptly at 4°C and valid for 4 weeks. In addition, keep unused slats in an aluminium foil bag containing desiccant seal tightly and store at 4°C.

Assay Protocol

1. **Add sample:** Set up the standard wells, sample wells and blank wells separately. Dilute the standard product and sample with diluent A, set standard holes 7 holes (S1-S7), add 100μL standard product of different concentration to each hole successively, add 100μL diluent A to each blank hole, and add 100 μL sample to each remaining hole.
2. **Add antibody:** Dilute antibody to working concentration with diluent A, add antibody 50μL per well.Sealing plate film sealing plate, 100-300rpm oscillation (ensure that each hole solution does not spill and can be fully mixed), temperature oscillation incubation for 1 hour.

   Note: 1. Complete steps 1 and 2 as soon as possible with an interval of no more than 10 minutes. The sequence of steps cannot be changed.

   2. Please refer to relevant literature to determine the approximate concentration of the protein to be detected in the sample. If the concentration is greater than or less than the maximum or minimum standard concentration of this kit, please carry out appropriate dilution or concentration before testing.
3. **Wash plate:** automatic washing plate or manual washing plate, each hole of the washing liquid is 300μL, injection and suction interval of 15-30 seconds.Wash the board five times.After the last plate washing is completed, the enzyme label plate is upside-down on the absorbent paper and patted dry properly, and the liquid in the hole is discarded;
4. **Add the enzyme-labeled antibody:** Dilute the enzyme-labeled antibody to the working concentration with diluant B, add the enzyme-labeled antibody working solution (prepared before use) 100μL in each well, replace the sealing plate with a new sealing plate, shake at 100-300rpm (ensure that the solution does not spill in each well and can be fully mixed), and incubate at room temperature for 30 minutes;
5. **Plate washing:** Repeat step 3.
6. **Add TMB Substrate:** Add TMB substrate solution 90μL to each hole, replace the new sealing plate film, and color rendering at room temperature (the reaction time should be controlled within 10-30

minutes, not more than 30 minutes.When the first 3-4 holes of the standard product have obvious gradient blue, the gradient of the last 3-4 holes is not obvious, it can be terminated);

7. **Add Stop Solution:** Add termination solution 50μL to each well to terminate the reaction, and the blue immediately turns to yellow.The addition sequence of the termination solution should be as close as possible to the addition sequence of the substrate solution.If the color is uneven, please gently shake the label plate to make the solution evenly mixed;
8. **Reading:** .After ensuring that there are no water droplets at the bottom of the enzyme label plate and no bubbles in the hole, the detection wavelength of 450nm is used to read the value within 10 minutes.It is recommended to use dual wavelengths, namely the detection wavelength of 450nm, the reference wavelength, or the correction wavelength of 630nm to read the value at the same time. Using only 450nm will reduce the accuracy.

Note

1. Store kits according to instructions. Gradually equilibrate samples to room temperature for 20-30 minutes before beginning assay.
2. The standards are lyophilised powders. The diluent volume and ratio should strictly follow the kit instructions. To ensure the accuracy of the standards, please do not reuse if they are left over.
3. The 25x Wash Buffer may crystallise at low temperatures. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved before prepare to working solution.
4. TMB is Hazardous. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
5. Stop Solution contains acid, care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
6. An intense aqua blue color indicates that the TMB Sbustrate is contaminated, do not use it.
7. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
8. All reagents must be at room temperature (25-28 ℃). Temperature below 25 ℃ will result in a significant decrease in the absorbance of the final detection.
9. Repeat wells are recommended for standards and samples to ensure confidence in the test results.
10. Do not expose kit reagents to strong light during storage or incubation.
11. Mix gently after sample addition to avoid foaming.
12. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
13. For research use only. Not for use in diagnostic or therapeutic procedures.
14. To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips for each transfer.
15. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffers. Do not put the absorbent paper directly into the wells to absorb water.

Results Analysis

1. **Results calculation**
   a) It is recommended to calculate the average absorbance values for each set of duplicate standards and samples to obtain more accurate results.
   b) Create a standard curve with curve-fitting statistical software by plotting the standard concentration on the abscissa against the OD value on the ordinate. the closer the correlation

coefficient R value is to 1, the better the fitting effect is. The best fitting curve was determined by regression analysis.

c) The sample concentration is calculated by substituting the OD value. If the test sample was diluted, multiply the appropriate dilution factor for actual concentration.

d) The standard curve can be linearised by taking a logarithmic fit to the concentration values and OD values. This process may result in more sample concentrations, but the accuracy of the data will be reduced.

2. **Typical data**

Perform a standard curve with each assay. The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). The standard curves provided in the instructions are for reference only.

ng/mL	OD		Average
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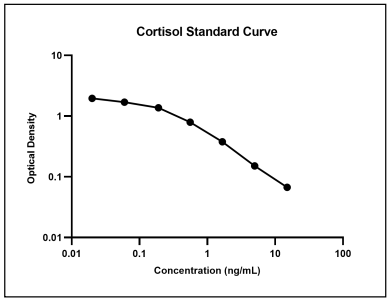
0.06	1.709	1.681	1.695
0.02	1.923	1.986	1.955
0	2.219	2.203	2.211



Test Protocol Summary

1. Prepare standards, reagents and samples according to instructions before the experiment.
2. Add sample (standard product or sample) 100μL/ well, then add antibody working solution 50μL/ well, and add sample continuously within 10 minutes.After mixing, shake at room temperature for 1 hour;
3. After washing for 5 times, pat dry, add enzyme-labeled antibody working solution 100μL/ well, and shake at room temperature for 30 minutes;
4. Wash for 5 times and pat dry, add TMB substrate 90μL/ well, incubate at room temperature for 10-30 minutes away from light;
5. Add termination solution 50μL/ well;
6. The OD value is detected at 450 nm wavelength within 10 minutes, with a reference wavelength of 630 nm.

Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S7	S7										
B	S6	S6										
C	S5	S5										
D	S4	S4										
E	S3	S3										

删除[a]: **Sensitivity**

The lowest detectable concentration of Cortisol is 8.6pg/mL(the average of three independent experiments).This value is the average OD value measured by 20 blank holes plus the concentration value corresponding to twice SD

Precision

Precision is expressed as the coefficient of variation(CV) of the measured values of the samples. CV（%）=(SD/Mean)×100.

Intra-assay Precision: 3 samples with low, mid and high level were assayed 20 times in multiple assays to determine precision between assays. Intra-assay Precision: CV<9%.

Inter-assay Precision: 3 samples with low, mid and high level were assayed in replicates of 8 to determine precision within an assay. Inter-assay Precision: CV<15%.

Spike recovery

A certain amount of Cortisol (labeled samples) was added to 5 sets of normal human serum, and the mean value was measured repeatedly, and the recovery rate was calculated (the ratio of measured value to theoretical value) for the serum without Cortisol.Recovery rates range from 80% to 107%, with an average recovery rate of 92%.

Linearity of dilution

A certain amount of Cortisol (labeled samples) was added to five sets of human serum and diluted in a series within the kinetic range of a standard curve to evaluate the linearity of the test.The linear range is the ratio of Cortisol content measured to theoretical value in a diluted sample.

Dilution
Mean(%)
Range(%)

1:2
93
85-108

1:4
95
89-101

1:8
108
94-118

1:16
105
99-115

Sample values

The serum/plasma samples of 30 healthy volunteers were analyzed with this kit. The drug use history of the volunteers was unknown, and all samples were positive.

F	S2	S2										
G	S1	S1										
H	S0	S0										

删除[a]: V1

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Servicebio® ELISA Stop Solution

Cat. #: G0027

Product Information

Product Name	Cat. No.	Spec.
ELISA Stop Solution	G0027-100ML	100 mL

Product Description/Introduction

This product is used to terminate the colour development reaction in ELISA experiments.

Stroage and Shipping Conditions

Ship and store at room temperature, valid for 24 months.

Product Components

Component	G0027-100ML
ELISA Stop Solution	100 mL
Manual	1 PC

Instructions for use

During the ELISA experiment, after adding TMB substrate to the plate to develop the colour to the expected shade, add 50 μL of stop solution per well to terminate the reaction. The order of addition of the stop solution should be the same as that of TMB substrate as far as possible. In case of uneven colour, please shake the plate gently to mix the solutions evenly. After making sure that there are no water droplets on the bottom of the plate and no air bubbles in the wells, measure the absorbance value at 450 nm within 30 minutes.

Note

This product is an acidic solution, please pay attention to proper protection when handling and avoid direct contact of the reagent with skin and eyes. In case of inadvertent contact, please immediately wash with plenty of water and seek medical advice.

For research use only. Not for use in diagnostic or therapeutic procedures!

Servicebio® Rapid Lentivirus Titration Cassette (Colloidal Gold)

Cat. #: G1804

Product Information

Product Name	Cat. No.	Spec.
Rapid Lentivirus Titration Cassette (Colloidal Gold)	G1804-10T	10 T

Product Description

This product is a semi-quantitative HIV-1 P24 protein assay card based on colloidal gold lateral chromatography that can be used for rapid assessment of the packaging efficiency of relevant lentivirus vectors and pseudoviruses (the P24 protein is the most abundant marker protein in the lentiviral capsid). The product is a rapid detection of free capsid protein P24 secreted into the lentiviral packaging supernatant using colloidal gold immunochromatography as a sensitive, indirect surrogate indicator of viral yield. The detection of capsid protein P24 enables a rapid determination of whether the lentiviral titer in the packaging solution has met the harvest needs. This product requires only 8-12 minutes to obtain results, which is a significant advantage over traditional transfection methods that take 2-3 days.

P24 protein concentration in relation to lentivirus titer:

P24 concentration	500 ng/mL	100 ng/mL	50 ng/mL	10 ng/mL	1 ng/mL
LPs/mL	6.25×10^{9}	1.25×10^{9}	6.25×10^{8}	1.25×10^{8}	1.25×10^{7}
TU/mL	$6.25\times10^{6-7}$	$1.25\times10^{6-7}$	$6.25\times10^{5-6}$	$6.25\times10^{5-6}$	$6.25\times10^{4-5}$

There are approximately 2000 P24 protein molecules in a Lentiviral Particle (LP) and normally 1 TU (Transducing Unit) of infectious virus vector for every 100-1000 LPs; a virus titer of at least 1 x 10^{6} TU/mL is required for certain types of cells to be transfected. (usually concentrated at around 150 times).

Result of lentivirus titer fast detection card (colloidal gold method) for 10 min:

Dilute P24 protein to 500 ng/mL, 100 ng/mL, 50 ng/mL, 10 ng/mL, 1 ng/mL and 0, then drop 80 μ L of diluent into the loading wells, place it flat on the table, stand for 8-12 minutes to observe the results.

Storage and Shipping Conditions

Ship and store at room temperature, valid for 12 months.

Product Components

Component Number	Component	G1804-10T
G1804-10T	Rapid Lentivirus Titration Cassette (colloidal gold)	10 T
Manual		1 pc

Assay Protocol

1. Open the package and take out the detection card, place it flat on the table.
2. Pipette 80~100 μL of culture supernatant from cells (such as 293 cells) packaged with lentivirus, and drop to the loading wells.
3. Stand for 8~12 minutes to obtain results, and they are invalid after 15 minutes.

Result Judgment

1. In the visual window, both the quality control line (C) and the test line (T) are coloured as positive.
2. The quality control line (C) is colored, the test line (T) is not colored, and the test result is negative.
3. The quality control line (C) is not colored. No matter whether the detection line (T) is colored or not, the result is invalid.
4. The colour of the (T) line varies with the content of P24, the higher the content, the darker the colour; the lower the content, the lighter the colour.

Note

1. If the color of tissue culture medium causes high background, dilute the sample with PBS or culture medium before chromatography.
2. It is not recommended to freeze the test card.
3. Use as soon as possible after opening.

Servicebio® His-tag Rapid Assay (Colloidal Gold)

Cat. #: G1906

Product Information

Product Name	Cat. No.	Spec.
His-tag Rapid Assay (colloidal gold)	G1906-10T	10 T

Product Description/Introduction

It is a semi-quantitative His protein test card based on the principle of colloidal gold lateral chromatography and the principle of competitive assay for the rapid detection of His-tagged proteins in cell culture supernatant, cell/bacterial lysate or protein purification.

The product mainly consists of a sample pad, binding pad, chromatography membrane, absorbent paper, and backing plate. Goat anti-mouse IgG polyclonal antibody (Control Line) and His-tagged protein (Test Line) were immobilized on the chromatographic membrane; colloidal gold-labeled His-tagged antibody was immobilized on the binding pad. When a drop of the sample to be measured is added to the sample pad, the liquid moves toward the binding pad by capillary action. If the sample does not contain His-tagged protein, the gold-labeled anti-His-tagged antibody on the binding pad binds to the His-tagged protein immobilized at the Test Line to form an "Au-anti-His-tagged antibody-His-tagged protein" complex, resulting in a red line at the Test Line; if His-tagged protein is contained in the test sample, His-tagged protein will combine with the gold standard anti-His-tagged antibody on the binding pad to form an "Au-anti-His-tagged antibody-His-tagged protein" complex, and the unreacted gold standard anti-His-tagged antibody on the binding pad will react with His-tagged protein on the Test Line, and a red line will appear on the Test Line. As the content of His-tagged protein in the sample under test increases, the coloration of the Test Line gradually decreases until it is not colorful at all. Regardless of the presence of His-tagged proteins in the sample being examined, the gold-labeled anti-His-tagged antibody on the binding pad will be chromatographed upward to the quality Control Line, where it reacts with the goat anti-mouse IgG to appear as a red line. The red line at Control Line is the criterion for determining whether the chromatography process is normal.

Storage and Shipping Conditions

Ship and store at room temperature, valid for 12 months.

Product Components

Component Number	Component	G1906-10T
G1906-10T	His-tag Rapid Assay (colloidal gold)	10 T
Manual		1 pc

Assay Procedures/Protocols

1. Open the package to take out the detection card and lay it flat on the lab bench.
2. Pipette 80 μL of culture supernatant, cell/bacterial lysis product, or post-protein purification sample dropwise into the sample hole.
3. Wait 8-10 minutes for the color bands to appear, it is invalid after 15 minutes.

Results Judgement

1. Negative result: Both the control line and the test line are coloured, with the test line showing the

darkest colour.

2. Positive result: The control line is coloured, the test line is weakly coloured or not coloured.
3. Invalid result: When the control line is not coloured, the test should be repeated whether the test line is coloured.

Note: When the sample does not contain His-tag protein, the color of the test line is darkest, and gradually decreases as the amount of His-tag protein in the sample increases, until the colour disappears.

Results Display

Take out the detection card and lay it flat on the lab bench. Dilute the His-tagged protein with PBS to 5 μg/mL, 20 μg/mL and 50 μg/mL respectively, and then add 80 μL of each dilution to the sample hole and leave it for 8 min, observe the results.

Results of His-tag Rapid Assay (colloidal gold) for 8 min

Sensitivity

The lower limit of detection is 5 μg/mL, when the His-tag protein content in a sample is below 5 μg/mL, it is not easily distinguish with the naked eye.

Note

1. If the colour of the sample causes a high background, the sample can be diluted with PBS or culture medium before chromatography.
2. Before testing, the refrigerated samples must be restored to room temperature, and the frozen samples must be completely melted, mixed, and returned to room temperature before testing. If the samples show obvious turbidity, please centrifuge and take the supernatant for testing.
3. For the accuracy of the results, a negative control and positive control should be set up, and it is recommended that the control sample is the same matrix as the sample to be tested.
4. This test card is not recommended for freezing.
5. Once opened please use quickly.
6. Samples containing imidazole are not suitable for this product

Flag-tag Rapid Assay (Colloidal Gold)

Cat.No. : G1907-10T

Brand : Servicebio

Spec.: 10 T

Product Introduction

Product Information

Product Name	Cat. No.	Spec.
Flag-tag Rapid Assay(colloidal gold)	G1907-10T	10 T

Product Description/Introduction

The Flag-tag Rapid Assay(colloidal gold) is a 10-minute dipstick lateral-flow assay to detectFlag -tagged proteins in cell culture and lysates during protein expression or inpurified protein preparations . The assay is performed by simply applying the lateral flow strip into the sample of properly diluted tissue culture supernatant or lysate and visualizing via a loss of red bands in the test line section.

The product mainly consists of a sample pad, binding pad, chromatography membrane, absorbent paper,andbacking plate.In a negative result where noFlag -tagged protein is present or concentration ofFlag-tagged protein is below detectable levels, the gold-conjugated capture antibodies will bind to theFlag -tagged protein antigen embedded on the test lines and formavisible red line. In both positive and negative tests, the goldconjugated anti-Flag capture antibodies will bind to the control antibodies at the control line. In a positive test where the sample containsFlag -tagged proteins, the gold-conjugated antibodies embedded in the sample pad will bind to the availableFlag -tag proteins in the sample and therefore not bind to theFlag-tagged protein antigens immobilized on the test lines. As the concentration increases, thecolor of test line will decrease until disappear. The concentration of theFlag-tagged proteins is inversely related to thecolor of test line appearing on the strip.

Storage and Shipping Conditions

Ship and store at room temperature, valid for 12 months.

Product Components

Component Number	Component	G1907-10T
G1907-10T	Flag-tag Rapid Assay(colloidal gold)	10 pc
Manual		1 pc

Servicebio® HA-tag Rapid Assay (Colloidal Gold)

Cat. #: G1908

Product Information

Product Name	Cat. No.	Spec.
HA-tag Rapid Assay (colloidal gold)	G1908-10T	10 T

Product Description/Introduction

It is a semi-quantitative HA protein assay card based on colloidal gold lateral chromatography and the principle of competitive assay for rapid detection of HA tagged proteins in cell culture supernatants, cell/bacterial lysate or after protein purification.

The product mainly consists of a sample pad, binding pad, chromatography membrane, absorbent paper, and backing plate.Goat anti-mouse IgG polyclonal antibody (Control Line) and HA-tagged protein (Test Line) were immobilized on the chromatographic membrane; colloidal gold-labeled HA-tagged antibody was immobilized on the binding pad. When a drop of the sample to be measured is added to the sample pad, the liquid moves toward the binding pad by capillary action. If the sample does not contain HA-tagged protein, the gold-labeled anti-HA-tagged antibody on the conjugate pad binds to the HA-tagged protein immobilized at the Test Line to form an "Au-anti-HA-tagged antibody-HA-tagged protein" complex, resulting in a red line at the Test Line; if the tested sample contains HA-tagged protein, the HA-tagged protein binds to the gold standard anti-HA-tagged antibody on the binding pad to form an "Au-anti-HA-tagged antibody-HA-tagged protein" complex, and the unreacted gold standard anti-HA-tagged antibody on the binding pad reacts with the HA-tagged protein on the Test Line, a red line appears on the Test Line as the content of HA-tagged protein increases, the coloration of the Test Line gradually decreases until it is completely colorless. Regardless of the presence of HA-tagged proteins in the sample being examined, the gold-labeled anti-HA-tagged antibody on the binding pad will be chromatographed upward to the control line, where it reacts with the goat anti-mouse IgG to appear as a red line. The red line at control line is the criterion for determining whether the chromatography process is normal.

Storage and Shipping Conditions

Ship and store at room temperature, valid for 12 months.

Product Components

Component Number	Component	G1908-10T
G1908-10T	HA-tag Rapid Assay (colloidal gold)	10 T
Manual		1 pc

Assay Procedures/Protocols

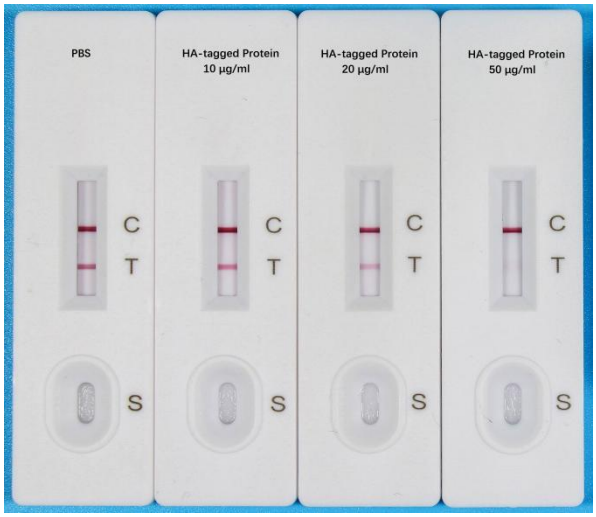
1. Open the package to take out the detection card and lay it flat on the lab bench.
2. Pipette 80 μL of culture supernatant, cell/bacterial lysis product, or post-protein purification sample dropwise into the sample hole.
3. Wait 8-10 minutes for the color bands to appear, it is invalid after 15 minutes.

1. Negative result: Both the control line and the test line are coloured, with the test line showing the darkest colour.
2. Positive result: The control line is coloured, the test line is weakly coloured or not coloured.
3. Invalid result: When the control line is not coloured, the test should be repeated whether the test line is coloured.

Note: When the sample does not contain HA-tag protein, the color of the test line is darkest, and gradually decreases as the amount of HA-tag protein in the sample increases, until the colour disappears.

Results Display

Take out the detection card and lay it flat on the lab bench. Dilute the Flag-tagged protein with PBS to 10 μg/mL, 20 μg/mL and 50 μg/mL respectively, and then add 100 μL of dilution to the sample hole and leave it for 8 min, observe the results.



Results of HA-tag Rapid Assay (colloidal gold) for 8 min

Sensitivity

The lower limit of detection is 20 μg/mL, when the HA-tag protein content in a sample is below 20 μg/mL, it is not easily distinguish with the naked eye.

Note

1. If the colour of the sample causes a high background, the sample can be diluted with PBS or culture medium before chromatography.
2. For the accuracy of the results, a negative control and positive control should be set up, and it is recommended that the control sample is the same matrix as the sample to be tested.
3. This test card is not recommended for freezing.
4. Once opened please use quickly.

Servicebio® S-tag Rapid Assay (Colloidal Gold)

Cat. #: G1909

Product Information

Product Name	Cat. No.	Spec.
S-tag Rapid Assay (colloidal gold)	G1909-10T	10 T

Product Description/Introduction

It is a semi-quantitative S protein test card based on colloidal gold lateral chromatography and the principle of competitive assay for rapid detection of S-tagged proteins in cell culture supernatants, cell/bacterial lysate or protein purified.

The product mainly consists of a sample pad, binding pad, chromatography membrane, absorbent paper, and backing plate. Goat anti-mouse IgG polyclonal antibody (Control Line) and S-tagged protein (Test Line) were immobilized on the chromatographic membrane; colloidal gold-labeled S-tagged antibody was immobilized on the binding pad. When a drop of the sample to be measured is added to the sample pad, the liquid moves toward the binding pad by capillary action. If the sample does not contain S-tagged protein, the gold-labeled anti-S-tagged antibody on the binding pad binds to the S-tagged protein immobilized at the Test Line to form an "Au-anti-S-tagged antibody-S-tagged protein" complex, which results in the appearance of a red line at the Test Line; if S-tagged protein is contained in the sample, the S-tagged protein will bind with the gold standard anti-S-tagged antibody on the binding pad to form an "Au-anti-S-tagged antibody-S-tagged protein" complex, and the unreacted gold standard anti-S-tagged antibody on the binding pad will react with the S-tagged protein on the Test Line, and a red line will appear on the Test Line. As the content of S-tagged protein in the sample increases, the coloration of the Test Line gradually decreases until it becomes completely colorless.

Storage and Shipping Conditions

Ship and store at room temperature, valid for 12 months.

Product Components

Component Number	Component	G1909-10T
G1909-10T	S-tag Rapid Assay (colloidal gold)	10 T
Manual		1 pc

Assay Procedures/Protocols

1. Open the package to take out the detection card and lay it flat on the lab bench.
2. Pipette 80 μL of culture supernatant, cell/bacterial lysis product, or post-protein purification sample dropwise into the sample hole.
3. Wait 8-10 minutes for the color bands to appear, it is invalid after 15 minutes.

Results Judgement

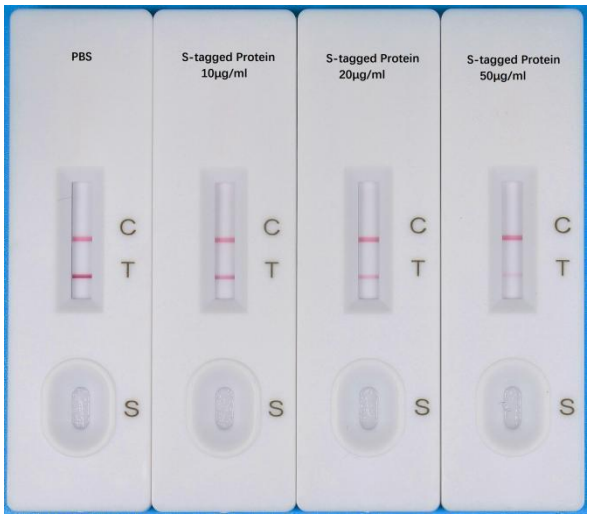
1. Negative result: Both the control line and the test line are coloured, with the test line showing the darkest colour.
2. Positive result: The control line is coloured, the test line is weakly coloured or not coloured.
3. Invalid result: When the control line is not coloured, the test should be repeated whether the test line is

coloured.

Note: When the sample does not contain S-tag protein, the color of the test line is darkest, and gradually decreases as the amount of S-tag protein in the sample increases, until the colour disappears.

Results Display

Take out the detection card and lay it flat on the lab bench. Dilute the Flag-tagged protein with PBS to 10 μg/mL, 20 μg/mL and 50 μg/mL respectively, and then add 100 μL of dilution to the sample hole and leave it for 8 min, observe the results.



Results of S-tag Rapid Assay (colloidal gold) for 8 min

Sensitivity

The lower limit of detection is 20 μg/mL, when the S-tag protein content in a sample is below 20 μg/mL, it is not easily distinguish with the naked eye.

Note

1. If the colour of the sample causes a high background, the sample can be diluted with PBS or culture medium before chromatography.
2. For the accuracy of the results, a negative control and positive control should be set up, and it is recommended that the control sample is the same matrix as the sample to be tested.
3. This test card is not recommended for freezing.
4. Once opened please use quickly.

V5-tag Quick Detection Card (Colloidal Gold Method)

Cat.No. : G1910-10T

Brand : Servicebio

Spec.: 10 T （V5-tag）

Product Introduction

Product Information

Product Name	Cat.No.	Spec.
V5-tag Quick Detection Card (Colloidal Gold Method)	G1910-10T	10T

Descirption/Introduction

This product is based on the principle of colloidal gold lateral chromatography and uses the principle of competitive method to quickly detect the content of V5 label protein in cell culture supernatant, cell/bacterial lysis product or protein purification. It is a semi-quantitative V5 protein detection card.

The main structure of this product is composed of sample pads, binding pads, chromatography films, absorbent paper, back plates, and other structures. Goat anti mouse IgG polyclonal antibody (quality control line C) and V5 label protein (detection line T) were fixed on the chromatographic membrane; A colloidal gold labeled V5 labeled antibody is fixed on the binding pad. When the sample to be tested is dripped onto the sample pad, the liquid moves towards the binding pad through capillary action.If the tested sample does not contain V5 label protein, the gold labeled anti V5 label antibody on the binding pad combines with the V5 label protein fixed at the test line T to form a "Au anti V5 label antibody V5 label protein" complex, resulting in a red band appearing at the test line T;If the tested sample contains V5 label protein, the V5 label protein binds to the gold labeled anti V5 label antibody on the binding pad to form a "Au anti V5 label antibody V5 label protein" complex. The unreacted gold labeled anti V5 label antibody on the binding pad will react with the V5 label protein on the test line T. A red band appears on the T line. As the content of V5 label protein in the tested sample increases, the color of the T line gradually decreases until it completely disappears.Regardless of whether there is a V5 labeled protein in the tested sample, the gold labeled anti V5 labeled antibody on the binding pad will be chromatographed upward to quality control line C, and a red band will appear in the reaction with sheep anti mouse IgG. The red bands shown on quality control line C are the criteria for determining whether the chromatographic process is normal.

Storage and Handling Conditions

Transported at room temperature, stored at room temperature, and valid for 12 months.

Component

Component Number	Component	G1910
G1910-10T	V5-tag Quick Detection Card (Colloidal Gold Method)	10T
manual		1

Assay Protocol / Procedures

1. Open the package and take out the detection card, and place it flat on the test bench;

2. Use a micropipette to absorb 80~100 μ L of culture supernatant, cell/bacterial lysis products or protein purified samples, and drop them into the sampling well;

3. Read the results after standing for 8~10min, and the interpretation results are invalid after 10min.

Result Judgment :

1. Negative results: In the visual window, both the quality control line C and the detection line T display color, and the T line displays the deepest color at this time;

2. Positive results: Quality control line C develops color, while test line T develops weak or no color;

3. Invalid result: The quality control line C does not display color, regardless of whether the test line T displays color, at this time, it needs to be retested;

Note: When the sample does not contain V5-tag protein, the test line T exhibits the deepest color development. As the content of V5-tag labeled protein in the sample increases, the color development of the test line T gradually decreases until the color development disappears.

Effect display:

Take out the test card, lay it flat on the experimental table, and dilute the V5 labeled protein with PBS to 5 μg/mL、10 μg/mL、20 μg/mL, then take 100 each μ L of diluent was added to the sampling hole and allowed to stand for 8 minutes. The results were observed.

Effect picture of V5-tag rapid detection card (colloidal gold method) for 10 min

Sensitivity:

The minimum detectable concentration is 10 μg/mL, when the content of V5-tag protein in the sample is lower than 5 μg/mL, it is difficult to distinguish with the naked eye.

Note:

1. If the sample chromatography background is high, PBS can be used to dilute the sample for chromatography;

2. In order to ensure the accuracy of the results, the customer needs to set up a negative control and a positive control. (It is recommended that the control sample and the sample to be tested be the same matrix.)

3. The reagent card is not recommended to be frozen;

4. Use as soon as possible after opening.

Servicebio® Sumo-tag Quick Detection Card (Colloidal Gold Method)

Cat. No.: G1911

Product information

Product name	Cat.No.	Spec.
Sumo-tag Quick Detection Card (Colloidal Gold Method)	G1911-10T	10T

Descirption/Introduction

This product is based on the principle of colloidal gold lateral chromatography, using the principle of sandwich method to rapidly detect the content of Sumo label protein after cell culture supernatant, cell/bacterial lysis product or protein purification, is a semi-quantitative Sumo label protein detection card.

The main structure of this product is composed of sample pad, bonding pad,chromatographic film, absorbent paper, backplane and other structures. Sheep anti-mouse IgG polyclonal antibody (quality control line C) and anti-Sumo protein antibody 1 (detection line T) were fixed on the chromatographic membrane. Another anti-Sumo protein antibody labeled with colloidal gold is fixed on the binding pad. When the sample to be measured is added to the sample pad, the liquid moves in the direction of the absorbent paper through capillary action. If the sample contains Sumo label protein, it first binds to gold-labeled anti-SUMO label antibody 1 on the binding pad, and then continues to bind to antibody 2 fixed at the detection line T to form a "gold-antibody 1-protein-antibody 2" complex, resulting in a red band on the detection line T and color on the quality control line C; On the contrary, if the sample does not contain Sumo label protein, the detection line T does not show color, and the quality control line C shows color. The red band presented by the quality control line C is the standard to judge whether the chromatographic process is normal.

Storage and Handling Conditions

Transport at room temperature, store at room temperature, valid for 12 months.

Component

Component Number	Component	G1906
G1911-10T	Sumo-tag quick detection card	10T
Manual		1

Assay Protocol / Procedures

1. Open the package, take out the test card, and put it on the test bench;
2. Use a micropipette to absorb 80-100 μL culture supernatant, cell/bacterial cleavage product or protein purified sample, and drop it into the sample adding hole;
3. Stand for 10 to 15 minutes to read the result. 20 minutes later, the interpretation result is invalid.

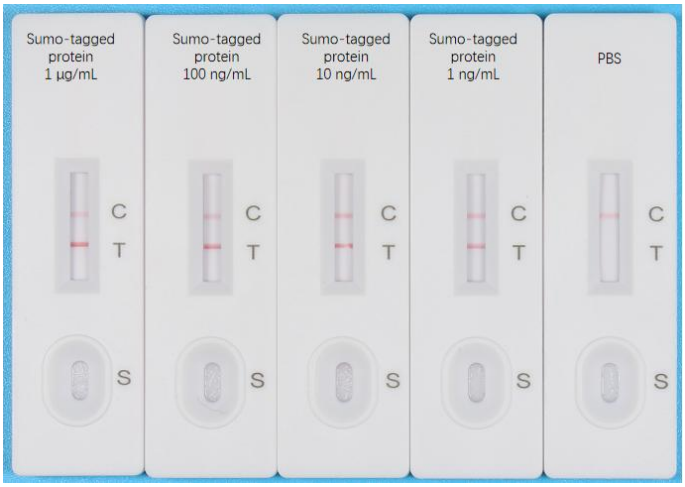
Result Judgment

1. Positive result: In the visual window, both the quality control line C and the detection line T show color;
2. Negative result: only quality control line C color;
3. Invalid resultquality control line C does not show color, no matter whether the detection line T shows color, it needs to be redetected at this time;

Note:When the Sumo-tag protein is 0.01 μg/mL-1 μg/mL, the color rendering intensity of the detection line T is positively correlated with the content of Sumo-tag tag protein in the detected sample.

Effect display

The detection card was removed and placed on the experimental table. The protein with Sumo label was diluted into 1 μg/mL, 100 ng/mL, 10 ng/mL and 1 ng/mL with PBS, respectively, and then 100 μL diluted drops were added to the sampling hole, and the results were observed after standing for 10 min.



The effect of Sumo-tag fast detection card (colloidal gold method) for 10 min

Sensitivity

The minimum detectable concentration is 1 ng/mL, and when the Sumo-tag protein content in the sample is less than 1 ng/mL, it is not easy to distinguish by naked eye.

Note

1. If the color of the sample causes the background to be high, the sample can be diluted with PBS or medium for chromatography.
2. Before testing,the refrigerated samples should be restored to room temperature, and the frozen samples should be completely melted, mixed, and returned to room temperature. If the sample is obviously cloudy,please remove the supernatant for testing.
3. For the accuracy of the results, the customer needs to set up a negative control, it is recommended that the control sample and the sample to be tested should be the same substrate.
4. The test reagent card is not recommended for freezing.
5. Use as soon as possible after opening.

Servicebio® Ultrafast Response Aqueous Adjuvant

Cat.#：G4704

Product information

product name	Identification of product	model
Ultrafast response aqueous Adjuvant	G4704-5ML	5 mL

Descirption/Introduction

This product is a water-soluble overspeed response adjuvant independently developed by Servicebio, with a unique formula and scientific modulation. Compared to traditional Freund's adjuvants, this product has the characteristics of low dosage, short cycle, fast efficacy, and minimal toxic side effects. Only 2 doses of immunity are needed to obtain high titers of antibodies. Rapid preparation of polyclonal antibodies and monoclonal antibodies suitable for mice, rats, and rabbits. This product does not require emulsification when used, it only needs to be mixed with the antigen at room temperature for simple immunization after 5 minutes.

Storage and Handling Conditions

Transported at 4℃, stored at 4℃, and valid for 12 months.

Assay Protocol / Procedures

Mix the antigen and this adjuvant in a 1:1 volume ratio, and after 5 minutes, inject the animal into the muscle (usually the thigh muscle). The interval between the first and second immunizations is 2 weeks. After 3 days of the second immunization, blood can be collected for potency testing, as well as subsequent cell fusion experiments.

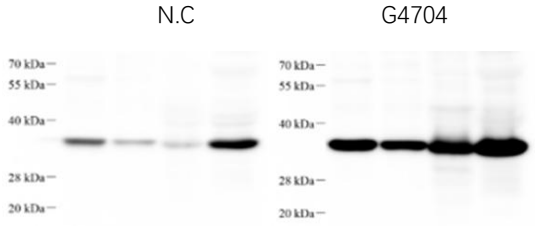
Effect display

PBS was used as a control (N.C) and this adjuvant G4704 was mixed with antigens for parallel immunization of two groups of mice. Blood samples were collected from the two groups of mice after 2 doses of immunization for serum potency testing. The immune enhancement effect of this adjuvant was significant:

N.C			G4704			
1	2	3	1	2	3	
1.217	1.212	1.196	1.214	1.254	1.237	1：1K
1.024	1.061	1.005	1.294	1.204	1.197	1：2K
0.803	0.912	0.829	1.201	1.208	1.139	1：4K
0.639	0.79	0.625	1.109	1.119	1.103	1：8K
0.46	0.495	0.399	1.081	1.075	1.004	1：16K
0.279	0.337	0.267	0.956	0.947	0.903	1：32K
0.192	0.23	0.144	0.775	0.787	0.735	1：64K
0.095	0.1	0.103	0.11	0.112	0.112	BLANK

Western blot endogenous detection of target proteins was performed on the antiserum of mice

immunized with 2 doses, and the immunomodulatory effect of this adjuvant was significant:



Product advantages

1. Traditional Freund's complete adjuvant (FCA) and incomplete adjuvant (FIA) belong to mineral oil adjuvants, with high toxicity and poor FIA activity. And this product can improve the antigen immune response of animals without serious adverse reactions, non-toxic side effects, safe and effective.
2. This product is water-soluble and can be used simply by mixing with antigens, avoiding the cumbersome emulsification process and the protein damage and antigen epitope loss caused by the heat generated during the emulsification process. Therefore, it is easy to screen and obtain antibodies with high affinity for conformational antigen epitopes.
3. This product has a fast immune response. Only 2 doses of immunization are needed to obtain high titer serum antibodies, which saves antigen usage and greatly shortens the antibody preparation cycle.

По вопросам продаж и поддержки обращайтесь:

Алматы (7273)495-231
Ангарск (3955)60-70-56
Архангельск (8182)63-90-72
Астрахань (8512)99-46-04
Барнаул (3852)73-04-60
Белгород (4722)40-23-64
Благовещенск (4162)22-76-07
Брянск (4832)59-03-52
Владивосток (423)249-28-31
Владикавказ (8672)28-90-48
Владимир (4922)49-43-18
Волгоград (844)278-03-48
Вологда (8172)26-41-59
Воронеж (473)204-51-73
Екатеринбург (343)384-55-89
Иваново (4932)77-34-06
Ижевск (3412)26-03-58
Иркутск (395)279-98-46
Казань (843)206-01-48
Калининград (4012)72-03-81
Калуга (4842)92-23-67
Кемерово (3842)65-04-62
Киров (8332)68-02-04
Коломна (4966)23-41-49
Кострома (4942)77-07-48
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