# Наборы для очистки белка

### Технические характеристики

**Виды товаров:** комплекты для очистки, колонки для очистки белков, протеаза, наборы для иммунопреципитации, магнитные шарики, очищенные антителами, очищенная антителами агароза, стойки для колонок для очистки белков, стрептавидин.

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### Servicebio<sup>®</sup> Streptavidin (HRP Conjugated)

### Cat #: G3431-200UL

### **Product Information**

Product Name	Cat. No.	Spec.
Streptavidin (HRP Conjugated)	G3431-200UL	200 µL

### Product Description/Introduction

Streptavidin (HRP conjugated) is a horseradish peroxidase (HRP) -labeled streptavidin, abbreviated as Streptavidin-HRP (SA-HRP), It is purified by chemical labeling method by conjugation of high purity horseradish peroxidase (HRP) to high purity Streptavidin, which has the lowest background and the highest sensitivity. It is mainly used for the detection of biotin-based labeled molecules (such as nucleic acids, proteins, antibodies, etc). Specific uses include: ELISA, Western, immunohistochemistry, in situ hybridization, Southern, Northern, etc.

### **Storage and Shipping Conditions**

Ship with wet ice; Store at -20°C, valid for 12 months.

### **Product Components**

Component Number	Component	G3431-200UL
G3431-1	Streptavidin (HRP conjugated)	200 µL
	Manual	

### Assay Protocol/Procedures

- 1. The protein concentration of this product is 1 mg/ mL, which is a ready-to-use product;
- 2. The recommended dilution ratio for various conventional uses of this product is shown in the following table. It is necessary to adjust the dilution ratio according to the specific situation in actual operation.

ELISA	Western	IHC/IC/ISH	Southern/Northern
1/2000-1/10000	1/2000-1/10000	1/200-1/500	1/2000-1/5000

### Note

1. For your safety and health, please wear safety glasses, gloves, or protective clothing.



## Servicebio<sup>®</sup> Recombinant Streptavidin (lyophilized Powder)

### Cat #: G3430

删除[Raisin]:

### **Product Information**

Product Name	Cat. No.	Spec.
Decombinent Strents idia (keepliked Douder)	G3430-5MG	5 mg
Recombinant Streptaviolin (lyophilized Powder)	G3430-100MG	100 mg

### Product Description/Introduction

Recombinant Streptavidin is a recombinant biotin-binding protein derived from Streptomyces avidinii expressed by Escherichia coli and contains His tag. Streptavidin and biotin have high affinity (Kd~10-15M) and low non-specific binding, so streptavidin-biotin system is widely used in various aspects of biological analysis. Streptavidin is a tetramer protein composed of four identical peptide chains with a mass of about 65kDa and can bind four biotin molecules. The recombinant streptavidin of this product has exactly the same properties as the natural streptavidin, and its purity is higher. It can not only be coupled with active substances such as enzyme and fluorescein, but also can be coupled with solid phase carriers such as magnetic beads and microspheres.

Source: E. coli strain carrying Streptomyces avidinii avidin gene;

Properties: white lyophilized powder

**Definition of enzyme activity:** One unit of the enzyme binds 1  $\mu$ g of biotin at room temperature.

Purity and activity: purity ≥95% detected by SDS-PAGE; Protein activity ≥15 U/mg.

### Storage and Shipping Conditions

Ship with wet ice; Store at 4°C, valid for 12 months; Store at -20°C, valid for 24 months.

### **Product Components**

Component Number	Component	G3430-5MG	G3430-100MG
G3430-1	Recombinant Streptavidin	5 mg	100 mg
	Manual		

### Assay Protocol/Procedures

Recombinant Streptavidin lyophilized powder resolution: Weigh appropriate amount of lyophilized powder, resolution with water or deionized water, 2-8 °C for short-term storage. It is recommended to freeze at -20 °C or lower after packaging, which can be stored for a long time and avoid repeated freezing and thawing.

### Note

1. For your safety and health, please wear safety glasses, gloves, or protective clothing.



## Servicebio<sup>®</sup> Recombinant Streptavidin (lyophilized Powder)

### Cat #: G3430

删除[Raisin]:

### **Product Information**

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Decombinent Strents idia (keepliked Douder)	G3430-5MG	5 mg
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Source: E. coli strain carrying Streptomyces avidinii avidin gene;

Properties: white lyophilized powder

**Definition of enzyme activity:** One unit of the enzyme binds 1  $\mu$ g of biotin at room temperature.

Purity and activity: purity ≥95% detected by SDS-PAGE; Protein activity ≥15 U/mg.

### Storage and Shipping Conditions

Ship with wet ice; Store at 4°C, valid for 12 months; Store at -20°C, valid for 24 months.

### **Product Components**

Component Number	Component	G3430-5MG	G3430-100MG
G3430-1	Recombinant Streptavidin	5 mg	100 mg
	Manual		

### Assay Protocol/Procedures

Recombinant Streptavidin lyophilized powder resolution: Weigh appropriate amount of lyophilized powder, resolution with water or deionized water, 2-8 °C for short-term storage. It is recommended to freeze at -20 °C or lower after packaging, which can be stored for a long time and avoid repeated freezing and thawing.

### Note

1. For your safety and health, please wear safety glasses, gloves, or protective clothing.



### Servicebio<sup>®</sup> Recombinant SUMO Protease (Ulp1, His-tag)

### Cat #:G3411-2000U

### **Product Information**

Product Name	Cat. No.	Spec.
Recombinant SUMO Protease (Ulp1, His-tag)	G3411-2000U	2000 U

### Product Description/Introduction

Recombinant SUMO Protease is a highly active cysteinyl protease also known as Ulp1 (ubiquitin-like protein-specific protease 1).SUMO Protease cleaves in a highly specific manner, recognizing the tertiary structure of the ubiquitin-like (UBL) protein, SUMO rather than an amino acid sequence. The optimal temperature for cleavage is  $30^{\circ}$ C; however, the enzyme is active over wide ranges of temperature (2- $30^{\circ}$ C) and pH (pH 6.0–10.0). In the actual operation, in order to preserve the structure and biological activity of the target protein, it is recommended to use Recombinant SUMO Protease at 4°C overnight. At the same time, adding appropriate concentration of DTT (0.5-2 mM) in the digestion system can also significantly improve the digestion efficiency.SUMO Protease, a highly active cysteinyl protease also known as Ulp, is a recombinant fragment of Ulp1 (Ubl-specific protease 1) from Saccharomyces cerevisiae. SUMO Protease cleaves in a highly specific manner, recognizing the tertiary structure of the ubiquitin-like (UBL) protein, SUMO rather than an amino acid sequence. The protease can be used to cleave SUMO from recombinant fusion proteins. The optimal temperature for cleavage is  $30^{\circ}$ C; however, the enzyme is active over wide ranges of temperature (2-30 °C) and pH (pH 6.0–10.0). Following digestion, SUMO Protease is easily removed from the cleavage reaction by affinity chromatography using the polyhistidine tag at the N-terminus of the protease.

Application: The Protease can be used to cleave SUMO from recombinant fusion proteins.

**Source:** Saccharomyces cerevisiae cysteine protease, It is purified from E. coli by affinity chromatography using the polyhistidine tag.

**Enzyme activity:** One unit of SUMO Protease cleaves  $\geq 85\%$  of 2µg control substrate in 1h at 30°C and pH 8.0.

**Inactivation or inhibition:** Concentrations of the imidazole higher than 150 mM can adversely affect the activity of the protease.

**Purity and concentration:** Purity  $\ge$  95% by SDS-PAGE, 10 U/µL.

**Recombinant SUMO protease storage solution:** 25 mM Tris-HCl, pH 8.0, 0.1% NP-40, 250 mM NaCl, 500 μM DTT, 50% (v/v) glycerol.

10×SUMO Buffer+Salt: 500 mM Tris-HCl, pH 8.0, 2% NP-40, 1.5 M NaCl, 10 mM DTT.

10×SUMO Buffer - Salt: 500 mM Tris-HCI, pH 8.0, 2% NP-40, 10 mM DTT.

### **Storage and Shipping Conditions**

Ship with wet ice; Store at -20°C; Valid for 12 months.

Component Number	Component	G3411-2000U
G3411-1	Recombinant SUMO Protease (His-tag) (10 U/ $\mu$ L)	200 µL
G3411-2	10×SUMO Buffer + Salt	1 mL



G3411-3	10×SUMO Buffer - Salt	1 mL
G3411-4	Control Protein	100 µL
	Manual	

### Assay Protocol/Procedures

1. Protease digestion system recommended:

Component	Volume
10×SUMO Buffer +/- Salt	5 μL
Target Proteins	20 µg
Recombinant SUMO Protease (His-tag) (10 U/µL)	1 μL
ddH <sub>2</sub> O	Το 50 μL

Note: For most fusion proteins, SUMO Protease functions optimally in a reaction mixture containing 150 mM NaCl; however, conditions may be optimized by varying the NaCl concentration from 0 mM to 300 mM.

- Place at 30°C for 1, 2 or 4 h. It can also digest at 4°C overnight (about 16 h) if the target protein is not stable at 30°C.;
- Remove 10 µL enzyme digestion product, for SDS-PAGE electrophoresis to evaluate the amount of enzyme digestion and reaction conditions.

### Note

- 1. Place in an ice box or ice bath when used, and separately store at -20°C immediately after use.
- 2. For your safety and health, please wear safety glasses, gloves, or protective clothing.

### Examples of enzyme digestion applications



Figure 1: Effect of recombinant SUMO Protease digestion of control substrate protein (SUMO+B fusion protein) (The enzyme digestion conditions are  $30^{\circ}$ C and pH 8.0, respectively for 0 min, 10 min, 20 min, 30 min, 1 h, 2 h and 4 h, corresponding to Lane 1-7 in turn, and the mass ratio of substrate and enzyme addin the enzyme digestion system is 10:1)



### Servicebio<sup>®</sup> Recombinant HRV 3C Protease (His-tag)

### Cat #: G3410-1000U

### **Product Information**

Product Name	Cat. No.	Spec.
Recombinant Protease (His-tag)	G3410-1000U	1000 U

### Product Description/Introduction

Recombinant HRV 3C Protease is a recombinant cysteine protease from human rhinovirus type 14 3C with His tag expressed in Escherichia coli. HRV 3C Protease cleaves with the recognition sequence L-E-V-L-F-Q-G-P (LeU-Glu-Val-Leu-Ph-Gln-Gly-Pro) or the core pentapeptide sequence L-F-Q-G-P (Leu-Ph-Gln-Gly-Pro) at low temperature between the Q (Gln) and G-P (Gly-Pro) residues. The high specificity and activity of the protease make it still active even at low temperature of 4°C.

**Application: R**emove MBP, GST, His or other tag proteins from fusion proteins by binding HRV 3C protease which contains His tag to the nickel column.

**Source:** Human Rhinovirus type 14 3C Protease, recombinant expressed in Escherichia coli, containing His tag.

**Enzyme activity :** It is defined as one active unit for cleaving more than 90% of 100  $\mu$ g substrate (protein containing HRV 3C Protease cleavage site) within 16 h at 4°C, pH 7.5.

**Inactivation or inhibition:** HRV 3C Protease activity was inhibited by 100 mM ZnCl2, 4 mM AEBSF, and 100  $\mu$ M Chymostatin.

Purity and concentration: Purity ≥95% by SDS-PAGE, 2 U/µL.

**Recombinant HRV 3C Protease storage solution:** 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 50% (v/v) glycerol, pH 8.0.

10×HRV 3C Buffer: 500 mM Tris-HCl, 1.5 M NaCl, 10 mM EDTA, 10 mM DTT, pH 7.5.

### **Storage and Shipping Conditions**

Ship with wet ice; Store at -20°C, valid for 12 months.

### Product Components

Component Number	Component	G3410-1000U
G3410-1	Recombinant HRV 3C Protease (His-tag) (2 U/ $\mu$ L)	500 μL
G3410-2	10×HRV 3C Buffer	3×1 mL
G3410-3	Control Protein	100 µL
Manual		

### Assay Protocol/Procedures

1. Protease digestion system recommended:

Component	Volume
10×HRV 3C Buffer	5 µL
Target Proteins	200 µg
Recombinant HRV 3C Protease (His-tag) (2U/µL)	1 µL



ddH <sub>2</sub> O	Το 50 μL

- 2. Place at  $4^{\circ}$ C overnight or for 16 h;
- 3. Remove 10 µL of enzyme digestion product for SDS-PAGE electrophoresis to evaluate the amount of enzyme digestion and reaction conditions.

### Note

- 1. Enzyme products should be placed in an ice box or ice bath when used and stored separately at -20°C immediately after use;
- 2. For your safety and health, please wear safety glasses, gloves, or protective clothing.

# M 1 2 3 4 5 6 -A+B -A

### Examples of enzyme digestion applications

Figure 1: Effect of HRV 3C Protease digestion of control substrate protein(A+B fusion protein, which contains HRV 3C Protease Enzyme cutting site) (The restriction conditions are 30°C, pH 7.5, 0 min, 10 min, 20 min, 30 min, 1 h, 2 h, respectively, corresponding to Lane: 1-6, the mass ratio of added substrate to enzyme in the enzymatic digestion system is 10:1)



## Servicebio<sup>®</sup> Hybrid Solution (50% Formamide)

### Cat. #: G3049-30ML

### **Product Information**

Product Name	Cat. No.	Spec.
Hybrid Solution (50% Formamide)	G3049-30ML	30 mL

### **Product Description**

The basic principle of In situ hybridization is that two single-stranded nucleotide fragments are combined by hydrogen bonds under appropriate conditions to form double-bond molecules such as DNA-DNA, DNA-RNA or RNA-RNA. Then DNA or RNA fragments with markers (such as radioisotope markers, fluorescent biotin markers, non-radioactive substance markers) are used as nucleic acid probes to hybridize with nucleic acid (RNA or DNA) fragments to be tested in tissue sections or cells. Finally, autoradiography, fluorescence signal detection, enzyme reaction color rendering and other methods are used for visualization. The presence and localization of the target RNA or DNA are observed with an optical microscope.

During In situ hybridization experiment, the hybridization solution is designed to provide the optimal microenvironment for nucleic acid hybridization. The main components generally contain formamide,  $5 \times$  Denhardt solution, SSC buffer system, specific nucleic acid probe. Formamide can reduce the stability of the double-stranded molecule and decrease the Tm value of the probe in a linear manner, that is, with the addition of 1% formamide, the Tm value decreases by about  $0.75 \sim 1.0^{\circ}$ C. For a typical probe, hybridization is performed at temperatures around Tm-20 °C , and the addition of formamide allows nucleic acid hybridization to be performed at lower temperature to ensure the stability of the probe molecules.

The main components of this product are 50% formamide,  $5 \times$  Denhardt solution and  $4 \times$  SSC. It can be used as prehybridization solution and hybridization solution in In situ hybridization experiment (additional probes need to be added by the user).

We also offer other formamide concentrations in hybridisation solutions, please refer to our website for details.

### **Storage and Shipping Conditions**

Ship at room temperature; Store at 2-8°C, away from direct sunlight, valid for 24 months.

### **Product Component**

Component Number	Component	Spec.
G3049-30ML	Hybrid Solution (50% Formamide)	30 mL

#### Note

1. Please tighten the bottle cap in time after use to prevent volatilization of active ingredients.

# Servicebio<sup>®</sup> Protein Gravity Purification Column (Affinity Chromatography Column Empty) Support

### Cat. #: G6063-20

### **Product Information**

Product Name	Cat. No.	Spec.
Protein Gravity Purification Column (Affinity		20 holos
Chromatography Column Empty) Support	G0003-20	20 Holes

### Product Description/Introduction

Purification of tagged recombinant proteins using gravity purification columns is simple and time-saving, and can be carried out simultaneously for gravity purification of one or more tagged recombinant proteins. At present, most laboratories use gravity purification columns to purify proteins are often homemade cartons or foam perforated fixed columns, this mode of fixing gravity purification columns are often unstable, not durable and so on. According to the experimental needs and combining the different specifications of protein gravity purification column size on the market, this product is designed to be compatible with the fixation of 1 mL, 3 mL, 6 mL, 12 mL, 30 mL, 60 mL gravity purification of the column can be compatible with a row of 10 holes), with height-adjustable and contains 20 holes, which can be carried out at the same time for the fixation of different specifications of the gravity purification columns. This protein gravity purification column holder is an easy-to-operate, time-saving, efficient, stable and durable protein gravity column purification device. The appearance of the product after assembly is shown as follows:



Product Appearance Diagram



### Note

- 1. This product is made of acrylic, pay attention to prevent the bracket falling from a high place
- 2. This product does not include the purification column shown in the schematic..

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# Servicebio<sup>®</sup> SweAgarose Protein A/G Antibody Purified Agarose

### Cat. #: G2236

### **Product Information**

Product Name	Cat. No.	Spec.
Swalarasa Dratain A/C Antibady Durified Agarosa	G2236-1ML	1 mL
Sweaglose Protein A/G Antibody Purned Agarose	G2236-5ML	5 mL

### Product Description/Introduction

Protein A is a cell wall surface protein found in *Staphylococcus aureus* with a molecular weight of 42kDa. Protein G is an immunoglobulin-binding protein expressed by *Streptococcal bacteria* (C or G).Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to agarose can be used for immunoprecipitation or antibody purification. Protein A agarose are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, while Protein G agarose are suitable for the immunoprecipitation of human IgG1, IgG2, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c polyclonal antibodies. (See Table I for specific information)

This product adopts the self-developed and produced Protein A/G labelled agarose, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.

Characteristics	Description
Product content	50%(v/v) agarose in specific protective buffer
Beads structure	6% cross-linked agarose
Coupled protein	Protein A/G
M.W.of protein	~25 kDa (Protein A/G)
Binding capacity	>1mg mouse antibody per ml beads
Specificity	Antibodies from different species, including mice, humans, rats, goats, sheep
эреспету	and cattle
Beads size	30~150 μm
	Elution with acid, or 1x SDS-PAGE loading buffer (reduced)
Flution mothed	Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~
Elution method	50kDa) and light chain (~ 25kDa) of the antibody will be denatured and
	released from the agarose beads.
Application	IP, Co-IP, Protein purification

### **Product Information**

### Storage and Shipping Conditions

Ship with wet ice; Store at 2-8°C, valid for 12 months.

Component Number	Component	G2236-1ML	G2236-5ML
G2236	SweAgrose Protein A/G Antibody Purified agarose	1 mL	5 mL
Manual		1	oc

### **Experiment preparation**

Antibody purification related reagent formulations refer to the following, the user can be adjusted according to specific experimental conditions.

Component	Reagent combination
Pinding Wash Puffor	PBST: 137 mM NaCl, 2.7 mM KCl, 10 mM Na <sub>2</sub> HPO <sub>4</sub> ,
binding wash buller	2.0 mM KH <sub>2</sub> PO <sub>4</sub> , 0.1% Tween-20
Elution Buffer	100 mM Gly, 0.1% Tween-20, pH 2.5
Neutralization Buffer	1.0 M Tris-HCl, pH 9.0
Preservation Buffer	PBST, 0.1%(v/v) Proclin 300

### Manual procedure (purification of mouse ascites IgG as an example)

- Sample processing: take 500 μL of ascites sample, add the binding wash buffer to make up 500 μL, if there are more protein precipitates in the sample, centrifuge the supernatant for experiments, which can improve the purity of the antibody.
- 2. Agarose pretreatment: Vortex the antibody purified agarose for 30 s to resuspend sufficiently, take 100 μL of 50% (v/v) SweAgarose Protein A/G Antibody Purified agarose in another new 1.5 mL EP tube, centrifuge at 6,000 x g for 30s at 4°C and discard the supernatant, wash it with 1 mL of binding washing buffer for twice, and then take the supernatant after magnetically aspirating.

Note: The amount of agarose can be adjusted according to the amount of antibody in the sample.

- 3. Antibody adsorption: add the sample processed in step 1 to the agarose in step 2, vortex and mix well, place the EP tube in a rotary mixer or manually turn the tube gently at room temperature (about 25°C) to make the agarose full contact with the sample, turn it over for 15 min, centrifuge at 6,000 x g for 30s at 4°C, and then discard the supernatant.
- 4. **Agarose washing:** add 1 mL of binding wash buffer to the EP tube, resuspend with shaking and then centrifuge at 6,000 x g for 30s at 4°C. Discard the supernatant and repeat the operation 3 times.
- 5. Antibody elution: Add 0.5~1.0 mL of eluent to the EP tubes with the agarose washed as described above, and resuspend the tubes rapidly by pipetting or vortexing, and then gently turn the tubes over in a turnover mixer or by hand at room temperature (about 25°C), centrifuge at 6,000 x g for 30s at 4°C after turning over for 10 min, and then collect the supernatant into new EP tubes.
- 6. Antibody neutralisation: add a certain amount of neutralisation solution to the antibody eluate in step 5, generally 1/10 of the antibody eluation volume (e.g., if the antibody eluate is 500 μL, the amount of neutralisation solution added is 50 μL), so that the pH value of the eluted antibody is maintained in a neutral environment, which is conducive to the maintenance of the biological activity of the antibody and the avoid antibody inactivation.
- 7. Post-treatment of Agarose: Wash the agarose twice with elution solution after use, centrifuge at 6,000 x g for 30s at 4°C, and discard the supernatant; then wash three times with binding washing solution, centrifuge at 6,000 x g for 30s at 4°C, and discard the supernatant; resuspend the agarose with 200 μL of preservation solution, and then store at 2~8°C.

### Note

- 1. Please read these instructions carefully before proceeding with antibody purification.
- 2. Do not freeze or centrifuge the agarose as this may cause irreversible aggregation of the agarose.

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
Human	lgG4	++++	++++
Human	IgA	++	-
	IgD	++	-
	lgE	++	-
	lgM	++	-
	lgG1	+	++++
	lgG2a	++++	++++
Mouse	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
	lgG1	-	+
Rat	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Table	1
-------	---

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++
-		

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding

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Version: V1.0-202308

### SweMagrose NH2



#### Product Introduction

#### **Product Information**

Product Name	Cat. No.	Spec.
SweMagrose NH-	G3658-1ML	1 mL
2	G3658-5ML	5 mL

#### **Product Description/Introduction**

Magnetic separation technology can easily separate or enrich antibodies, antigens, lectins, proteases, nucleic acids and cells while maintaining their biological activity. SweMagrose

NH2 perfectly fused superparamagnetic materials and polymer materials together by polymer polymerization technology to form a suspension of superparamagnetic iron oxide microspheres, which was coated with carboxyl groups. Compared with traditional magnetic beads, SweMagrose
NH2 has faster magnetic response, good dispersion, very low non-specific adsorption and more abundant binding sites. SweMagrose
NH2 can efficiently bind to a variety of biological ligands (oligonucleotides, peptides, proteins, drug molecules, etc.) under the action of special reagents (such as glutaraldehyde). It is a good basic material for subsequent treatment, such as coating, adsorption, chemical modification and so on.
Abundant binding sites: Enhances specific binding to ligand
High Magnetic Response: Faster magnetic response saves experiment time.

Superparamagnetic: good dispersion even after the disappearance of the external magnetic field.

Excellent dispersibility and resuspension: good dispersibility and resuspension ensure the stability of the experiment and facilitate the operation.

Excellent physicochemical stability: better guarantee of experimental reproducibility.

Component	SweMagrose NH <sub>2</sub>
Bead Diameter	30~150 μm
Surface amino content	60 µmol/mL gel
Preservation solution	20% ethanol solution
Magnetic core	Fe <sub>3</sub> 0 <sub>4</sub>
Shell layer	Agarose
Magnetization	Superparamagnetism

### SweMagrose NH2

	Consultation Cat.No.: G3658-1ML
accord a	Brand : Servicebio
Size 1 ml 312 2023 SG Hannard and	Spec.: 1 mL

Product Introduction			
Product Information			

Product Name	Cat. No.	Spec.
SweMagrose NH <sub>2</sub>	G3658-1ML	1 mL
	G3658-5ML	5 mL

#### **Product Description/Introduction**

Magnetic separation technology can easily separate or enrich antibodies, antigens, lectins, proteases, nucleic acids and cells while maintaining their biological activity. SweMagrose

NH2 perfectly fused superparamagnetic materials and polymer materials together by polymer polymerization technology to form a suspension of superparamagnetic iron oxide microspheres, which was coated with carboxyl groups. Compared with traditional magnetic beads, SweMagrose NH2 has faster magnetic response, good dispersion, very low non-specific adsorption and more abundant binding sites. SweMagrose NH2 can efficiently bind to a variety of biological ligands (oligonucleotides, peptides, proteins, drug molecules, etc.) under the action of special reagents (such as glutaraldehyde). It is a good basic material for subsequent treatment, such as coating, adsorption, chemical modification and so on.
Abundant binding sites:Enhances specific binding to ligand

High Magnetic Response: Faster magnetic response saves experiment time.

Superparamagnetic: good dispersion even after the disappearance of the external magnetic field.

Excellent dispersibility and resuspension: good dispersibility and resuspension ensure the stability of the experiment and facilitate the operation.

Excellent physicochemical stability: better guarantee of experimental reproducibility.

Component	SweMagrose NH <sub>2</sub>
Bead Diameter	30~150 μm
Surface amino content	60 µmol/mL gel
Preservation solution	20% ethanol solution
Magnetic core	Fe <sub>3</sub> 0 <sub>4</sub>
Shell layer	Agarose
Magnetization	Superparamagnetism



# Servicebio<sup>®</sup> SweMagrose Protein G Antibody Purified Magnetic Beads

### Cat. #: G3657

### **Product Information**

Product Name	Cat. No.	Spec.
Drotain A Antibody Durified Magnetic Peeds	G3657-1ML	1 mL
Protein A Antibody Purnied Magnetic beaus	G3657-5ML	5 mL

### Product Description/Introduction

Protein G is an immunoglobulin-binding protein expressed by Streptococcal bacteria (C or G). Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42kDa. Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to magnetic beads can be used for immunoprecipitation or antibody purification. while Protein G magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG2c polyclonal antibodies. Protein A magnetic beads are suitable for the immunoprecipitation of human IgG2b, IgC2b, IgC2b, IgC2b, IgC2b, IgC2b, IgC2b, IgC2b,

This product adopts the self-developed and produced Protein G protein-labelled magnetic beads, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.

### **Product Information**

Characteristics	Description
Product content	50 mg/ml magnetic beads in specific protective buffer
Magnetization	Superparamagnetic
Coupled protein	Protein A
M.W.of protein	~25 kDa (Protein A)
Binding capacity	>1mg Mouse IgG per mL beads
Specificity	antibodies from many different species, including mouse,human,rat,cow,goat and sheep
Beads size	30~150 μm
	Acid or SDS-PAGE loading buffer elution.
Elution method	Note: If elute with SDS-PAGE loading buffer,the light(~25 kDa) and heavy(~50
	kDa) chain of antibody will be denatured and release from the beads
Application	IP,Co-IP,Protein purification

### Storage and Shipping Conditions

Ship with wet ice; Store at 4°C, valid for 12 months.

Component Number	Component	G3657-1ML	G3657-5ML
G3657	SweMagrose ProteinG Antibody Purified Magnetic Beads	1 mL	5 mL
Manual		1pc	;

### **Experiment preparation**

Antibody purification related reagent formulations refer to the following, the user can be adjusted according to specific experimental conditions.

Component	Reagent combination	
Diadian Mash Duffer	PBST:137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4,	
Binding wash Buffer	2.0 mM KH2PO4, 0.1% Tween-20	
Elution Buffer	100 mM Gly, 0.1% Tween-20, pH 2.5	
Neutralization Buffer	1.0 M Tris-HCl, pH 9.0	
Preservation Buffer	PBST, 0.1%(v/v) Proclin 300	

### Manual procedure (purification of mouse ascites IgG as an example)

- 1. **Sample processing:** take 500 μL of ascites sample, add the binding wash buffer to make up 500 μL, if there are more protein precipitates in the sample, centrifuge the supernatant for experiments, which can improve the purity of the antibody.
- 2. Magnetic bead pretreatment: Vortex the antibody purified magnetic beads for 30 s to resuspend sufficiently, take 100 µL of 50% (v/v) SweMagrose Protein A Antibody Purified Magnetic Beads in another new 1.5 mL EP tube, magnetically aspirate it and discard the supernatant, wash it with 1 mL of binding washing buffer for twice, and then take the supernatant after magnetically aspirating. Note: The amount of beads can be adjusted according to the amount of antibody in the sample.
- 3. Antibody adsorption: add the sample processed in step 1 to the magnetic beads in step 2, vortex and mix well, place the EP tube in a rotary mixer or manually turn the tube gently at room temperature (about 25°C) to make the magnetic beads full contact with the sample, turn it over for 15 min, and then place it on the magnetic separator rack for 30 s, and then discard the supernatant.
- 4. **Magnetic bead washing:** add 1 mL of binding wash buffer to the EP tube, resuspend with shaking and then magnetically absorb for 30 s. Discard the supernatant and repeat the operation 3 times.
- 5. Antibody elution: Add 0.5~1.0 mL of eluent to the EP tubes with the magnetic beads washed as described above, and resuspend the tubes rapidly by pipetting or vortexing, and then gently turn the tubes over in a turnover mixer or by hand at room temperature (about 25°C), and then separate the tubes by magnetic suction after turning over for 10 min, and then collect the supernatant into new EP tubes.
- 6. Antibody neutralisation: add a certain amount of neutralisation solution to the antibody eluate in step 5, generally 1/10 of the antibody elution volume (e.g., if the antibody eluate is 500 μL, the amount of neutralisation solution added is 50 μL), so that the pH value of the eluted antibody is maintained in a neutral environment, which is conducive to the maintenance of the biological activity of the antibody and the avoid antibody inactivation.
- Post-treatment of magnetic beads: Wash the beads twice with elution solution after use, separate magnetically, and discard the supernatant; then wash three times with binding washing solution, separate magnetically, and discard the supernatant; resuspend the beads with 200 μL of preservation solution, and then store at 2~8°C.

### Note

- 1. Please read these instructions carefully before proceeding with antibody purification.
- 2. Do not freeze or centrifuge the beads as this may cause irreversible aggregation of the beads.
- 3. Manual operation requires the use of a magnetic separation frame.

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
Human	lgG4	++++	++++
Human	IgA	++	-
	lgD	++	-
	IgE	++	-
	lgM	++	-
	lgG1	+	++++
	lgG2a	++++	++++
Mouse	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
	lgG1	-	+
Rat	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Table 1	
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Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding

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# Servicebio<sup>®</sup> SweMagrose Protein G Antibody Purified Magnetic Beads

### Cat. #:G3657

### **Product Information**

Product Name	Cat. No.	Spec.
Drotain A Antibody Durified Magnetic Peeds	G3657-1ML	1 mL
Protein A Antibody Purified Magnetic Beads	G3657-5ML	5 mL

### Product Description/Introduction

Protein G is an immunoglobulin-binding protein expressed by Streptococcal bacteria (C or G). Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42kDa. Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to magnetic beads can be used for immunoprecipitation or antibody purification. while Protein G magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG2c polyclonal antibodies. Protein A magnetic beads are suitable for the immunoprecipitation of human IgG2b, IgC2b, IgC2b, IgC2b, IgC2b, IgC2b, IgC2b, IgC2b,

This product adopts the self-developed and produced Protein G protein-labelled magnetic beads, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.

### **Product Information**

Characteristics	Description
Product content	50 mg/ml magnetic beads in specific protective buffer
Magnetization	Superparamagnetic
Coupled protein	Protein A
M.W.of protein	~25 kDa (Protein A)
Binding capacity	>1mg Mouse IgG per mL beads
Specificity	antibodies from many different species, including mouse,human,rat,cow,goat and sheep
Beads size	30~150 μm
	Acid or SDS-PAGE loading buffer elution.
Elution method	Note: If elute with SDS-PAGE loading buffer,the light(~25 kDa) and heavy(~50
	kDa) chain of antibody will be denatured and release from the beads
Application	IP,Co-IP,Protein purification

### Storage and Shipping Conditions

Ship with wet ice; Store at 4°C, valid for 12 months.

Component Number	Component	G3657-1ML	G3657-5ML
G3657	SweMagrose ProteinG Antibody Purified Magnetic Beads	1 mL	5 mL
Manual		1pc	:

### **Experiment preparation**

Antibody purification related reagent formulations refer to the following, the user can be adjusted according to specific experimental conditions.

Component	Reagent combination
Binding Wash Buffer	PBST:137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4,
	2.0 mM KH2PO4, 0.1% Tween-20
Elution Buffer	100 mM Gly, 0.1% Tween-20, pH 2.5
Neutralization Buffer	1.0 M Tris-HCl, pH 9.0
Preservation Buffer	PBST, 0.1%(v/v) Proclin 300

### Manual procedure (purification of mouse ascites IgG as an example)

- 1. **Sample processing:** take 500 μL of ascites sample, add the binding wash buffer to make up 500 μL, if there are more protein precipitates in the sample, centrifuge the supernatant for experiments, which can improve the purity of the antibody.
- 2. Magnetic bead pretreatment: Vortex the antibody purified magnetic beads for 30 s to resuspend sufficiently, take 100 µL of 50% (v/v) SweMagrose Protein A Antibody Purified Magnetic Beads in another new 1.5 mL EP tube, magnetically aspirate it and discard the supernatant, wash it with 1 mL of binding washing buffer for twice, and then take the supernatant after magnetically aspirating. Note: The amount of beads can be adjusted according to the amount of antibody in the sample.
- 3. Antibody adsorption: add the sample processed in step 1 to the magnetic beads in step 2, vortex and mix well, place the EP tube in a rotary mixer or manually turn the tube gently at room temperature (about 25°C) to make the magnetic beads full contact with the sample, turn it over for 15 min, and then place it on the magnetic separator rack for 30 s, and then discard the supernatant.
- 4. **Magnetic bead washing:** add 1 mL of binding wash buffer to the EP tube, resuspend with shaking and then magnetically absorb for 30 s. Discard the supernatant and repeat the operation 3 times.
- 5. Antibody elution: Add 0.5~1.0 mL of eluent to the EP tubes with the magnetic beads washed as described above, and resuspend the tubes rapidly by pipetting or vortexing, and then gently turn the tubes over in a turnover mixer or by hand at room temperature (about 25°C), and then separate the tubes by magnetic suction after turning over for 10 min, and then collect the supernatant into new EP tubes.
- 6. Antibody neutralisation: add a certain amount of neutralisation solution to the antibody eluate in step 5, generally 1/10 of the antibody elution volume (e.g., if the antibody eluate is 500 μL, the amount of neutralisation solution added is 50 μL), so that the pH value of the eluted antibody is maintained in a neutral environment, which is conducive to the maintenance of the biological activity of the antibody and the avoid antibody inactivation.
- Post-treatment of magnetic beads: Wash the beads twice with elution solution after use, separate magnetically, and discard the supernatant; then wash three times with binding washing solution, separate magnetically, and discard the supernatant; resuspend the beads with 200 μL of preservation solution, and then store at 2~8°C.

### Note

- 1. Please read these instructions carefully before proceeding with antibody purification.
- 2. Do not freeze or centrifuge the beads as this may cause irreversible aggregation of the beads.
- 3. Manual operation requires the use of a magnetic separation frame.

Table 1	Т	a	b	le	1
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Species	Ig Protein A F		Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
	lgG4	++++	++++
Human	IgA	++	-
	IgD	++	-
	lgE	++	-
	lgM	++	-
Mouse	lgG1	+	++++
	lgG2a	++++	++++
	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
	lgG1	-	+
Rat	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	IgM	+/-	-

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++
0.	D.	

++++: Strong Bing

++~+++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding

For research use only	. Not for use	in diagnostic or	therapeutic	procedures!



# Servicebio<sup>®</sup> SweMagrose Protein A Antibody Purified Magnetic Beads

### Cat. #: G3656

### **Product Information**

Product Name	Cat. No.	Spec.
Drotain A Antibody Durified Magnetic Boods	G3656-1ML	1 mL
Protein A Antibody Purified Magnetic Beads	G3656-5ML	5 mL

### Product Description/Introduction

Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42kDa. Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to magnetic beads can be used for immunoprecipitation or antibody purification. Protein A magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, while Protein G magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c polyclonal antibodies. (See Table I for specific information)

This product adopts the self-developed and produced Protein A protein-labelled magnetic beads, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.

### **Product Information**

Characteristics	Description
Product content	50 mg/ml magnetic beads in specific protective buffer
Magnetization	Superparamagnetic
Coupled protein	Recombinant Protein A
M.W.of protein	~25 kDa (Protein A)
Binding capacity	>1mg Mouse IgG per mL beads
Specificity	antibodies from many different species, including
opeementy	mouse,human,rabbit,cow,goat and sheep
Beads size	30~150 μm
	Acid or SDS-PAGE loading buffer elution.
Elution method	Note: If elute with SDS-PAGE loading buffer, the light(~25 kDa) and heavy(~50
	kDa) chain of antibody will be denatured and release from the beads
Application	IP,Co-IP,Protein purification

### Storage and Shipping Conditions

Ship with wet ice; Store at 4°C, valid for 12 months.

Component Number	Component	G3656-1ML	G3656-5ML
G3656 SweMagrose Protein A Antibody Purified Magnetic Beads		1 mL	5 mL
Manual		1	.pc

### **Experiment preparation**

Antibody purification related reagent formulations refer to the following, the user can be adjusted according to specific experimental conditions.

Component	Reagent combination	
Pinding Wash Puffer	PBST: 137 mM NaCl, 2.7 mM KCl, 10 mM Na <sub>2</sub> HPO <sub>4</sub> ,	
Binding wash Butter	2.0 mM KH <sub>2</sub> PO <sub>4</sub> , 0.1% Tween-20	
Elution Buffer	100 mM Gly, 0.1% Tween-20, pH 2.5	
Neutralization Buffer	1.0 M Tris-HCl, pH 9.0	
Preservation Buffer	PBST, 0.1%(v/v) Proclin 300	

### Manual procedure (purification of mouse ascites IgG as an example)

- 1. **Sample processing:** take 500 μL of ascites sample, add the binding wash buffer to make up 500 μL, if there are more protein precipitates in the sample, centrifuge the supernatant for experiments, which can improve the purity of the antibody.
- 2. Magnetic bead pretreatment: Vortex the antibody purified magnetic beads for 30 s to resuspend sufficiently, take 100 µL of 50% (v/v) SweMagrose Protein A Antibody Purified Magnetic Beads in another new 1.5 mL EP tube, magnetically aspirate it and discard the supernatant, wash it with 1 mL of binding washing buffer for twice, and then take the supernatant after magnetically aspirating. Note: The amount of beads can be adjusted according to the amount of antibody in the sample.
- 3. Antibody adsorption: add the sample processed in step 1 to the magnetic beads in step 2, vortex and mix well, place the EP tube in a rotary mixer or manually turn the tube gently at room temperature (about 25°C) to make the magnetic beads full contact with the sample, turn it over for 15 min, place it on the magnetic separator rack for 30 s, and then discard the supernatant.
- 4. **Magnetic bead washing:** add 1 mL of binding wash buffer to the EP tube, resuspend with shaking and then magnetically absorb for 30 s. Discard the supernatant and repeat the operation 3 times.
- 5. Antibody elution: Add 0.5~1.0 mL of eluent to the EP tubes with the magnetic beads washed as described above, and resuspend the tubes rapidly by pipetting or vortexing, and then gently turn the tubes over in a turnover mixer or by hand at room temperature (about 25°C), separate the tubes by magnetic suction after turning over for 10 min, and then collect the supernatant into new EP tubes.
- 6. Antibody neutralisation: add a certain amount of neutralisation solution to the antibody eluate in step 5, generally 1/10 of the antibody elution volume (e.g., if the antibody eluate is 500 μL, the amount of neutralisation solution added is 50 μL), so that the pH value of the eluted antibody is maintained in a neutral environment, which is conducive to the maintenance of the biological activity of the antibody and the avoid antibody inactivation.
- 7. Post-treatment of magnetic beads: Wash the beads twice with elution solution after use, separate magnetically, and discard the supernatant; then wash three times with binding washing solution, separate magnetically, and discard the supernatant; resuspend the beads with 200 μL of preservation solution, and then store at 2~8°C.

### Note

- 1. Please read these instructions carefully before proceeding with antibody purification.
- 2. Do not freeze or centrifuge the beads as this may cause irreversible aggregation of the beads.
- 3. Manual operation requires the use of a magnetic separation frame.

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
Human	lgG4	++++	++++
пипап	IgA	++	-
	lgD	++	-
	IgE	++	-
	lgM	++	-
	lgG1	+	++++
	lgG2a	++++	++++
Mouse	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Table 1	
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Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding

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# Servicebio<sup>®</sup> SweMagrose Protein A Antibody Purified Magnetic Beads

### Cat. #: G3656

### **Product Information**

Product Name	Cat. No.	Spec.
Drotain A Antibody Durified Magnetic Boods	G3656-1ML	1 mL
Protein A Antibody Punned Magnetic Beads	G3656-5ML	5 mL

### Product Description/Introduction

Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42kDa. Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to magnetic beads can be used for immunoprecipitation or antibody purification. Protein A magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, while Protein G magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c polyclonal antibodies. (See Table I for specific information)

This product adopts the self-developed and produced Protein A protein-labelled magnetic beads, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.

### **Product Information**

Characteristics	Description	
Product content	50 mg/ml magnetic beads in specific protective buffer	
Magnetization	Superparamagnetic	
Coupled protein	Recombinant Protein A	
M.W.of protein	~25 kDa (Protein A)	
Binding capacity	>1mg Mouse IgG per mL beads	
Specificity	antibodies from many different species, including	
opeemeity	mouse,human,rabbit,cow,goat and sheep	
Beads size	30~150 μm	
	Acid or SDS-PAGE loading buffer elution.	
Elution method	Note: If elute with SDS-PAGE loading buffer,the light(~25 kDa) and heavy(~50	
	kDa) chain of antibody will be denatured and release from the beads	
Application	IP,Co-IP,Protein purification	

### Storage and Shipping Conditions

Ship with wet ice; Store at 4°C, valid for 12 months.

Component Number	Component	G3656-1ML	G3656-5ML
G3656 SweMagrose Protein A Antibody Purified Magnetic Beads		1 mL	5 mL
Manual		1	.pc

### **Experiment preparation**

Antibody purification related reagent formulations refer to the following, the user can be adjusted according to specific experimental conditions.

Component	Reagent combination	
Pinding Wash Puffer	PBST: 137 mM NaCl, 2.7 mM KCl, 10 mM Na <sub>2</sub> HPO <sub>4</sub> ,	
Binding wash Butter	2.0 mM KH <sub>2</sub> PO <sub>4</sub> , 0.1% Tween-20	
Elution Buffer	100 mM Gly, 0.1% Tween-20, pH 2.5	
Neutralization Buffer	1.0 M Tris-HCl, pH 9.0	
Preservation Buffer	PBST, 0.1%(v/v) Proclin 300	

### Manual procedure (purification of mouse ascites IgG as an example)

- 1. **Sample processing:** take 500 μL of ascites sample, add the binding wash buffer to make up 500 μL, if there are more protein precipitates in the sample, centrifuge the supernatant for experiments, which can improve the purity of the antibody.
- 2. Magnetic bead pretreatment: Vortex the antibody purified magnetic beads for 30 s to resuspend sufficiently, take 100 µL of 50% (v/v) SweMagrose Protein A Antibody Purified Magnetic Beads in another new 1.5 mL EP tube, magnetically aspirate it and discard the supernatant, wash it with 1 mL of binding washing buffer for twice, and then take the supernatant after magnetically aspirating. Note: The amount of beads can be adjusted according to the amount of antibody in the sample.
- 3. Antibody adsorption: add the sample processed in step 1 to the magnetic beads in step 2, vortex and mix well, place the EP tube in a rotary mixer or manually turn the tube gently at room temperature (about 25°C) to make the magnetic beads full contact with the sample, turn it over for 15 min, place it on the magnetic separator rack for 30 s, and then discard the supernatant.
- 4. **Magnetic bead washing:** add 1 mL of binding wash buffer to the EP tube, resuspend with shaking and then magnetically absorb for 30 s. Discard the supernatant and repeat the operation 3 times.
- 5. Antibody elution: Add 0.5~1.0 mL of eluent to the EP tubes with the magnetic beads washed as described above, and resuspend the tubes rapidly by pipetting or vortexing, and then gently turn the tubes over in a turnover mixer or by hand at room temperature (about 25°C), separate the tubes by magnetic suction after turning over for 10 min, and then collect the supernatant into new EP tubes.
- 6. Antibody neutralisation: add a certain amount of neutralisation solution to the antibody eluate in step 5, generally 1/10 of the antibody elution volume (e.g., if the antibody eluate is 500 μL, the amount of neutralisation solution added is 50 μL), so that the pH value of the eluted antibody is maintained in a neutral environment, which is conducive to the maintenance of the biological activity of the antibody and the avoid antibody inactivation.
- 7. Post-treatment of magnetic beads: Wash the beads twice with elution solution after use, separate magnetically, and discard the supernatant; then wash three times with binding washing solution, separate magnetically, and discard the supernatant; resuspend the beads with 200 μL of preservation solution, and then store at 2~8°C.

### Note

- 1. Please read these instructions carefully before proceeding with antibody purification.
- 2. Do not freeze or centrifuge the beads as this may cause irreversible aggregation of the beads.
- 3. Manual operation requires the use of a magnetic separation frame.

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
Human	lgG4	++++	++++
Human	IgA	++	-
	lgD	++	-
	IgE	++	-
	lgM	++	-
	lgG1	+	++++
	lgG2a	++++	++++
Mouse	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Table 1	
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Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding

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# Servicebio<sup>®</sup> SweMagrose IDA-Co Magnetic Agarose Beads for His-Tag Protein Purification

### Cat. #:G3655

### **Product Information**

Product Name	Cat. No.	Spec.
SweMagrose IDA-Co Magnetic Agarose Beads for	G3655-1ML	1 mL
His-Tag Protein Purification	G3655-5ML	5 mL

### Product Description/Introduction

This product is prepared from agarose magnetic beads modified by IDA and chelating nickel ions. It can efficiently and quickly purify the histidine-tagged proteins in biological samples without centrifugal filtration. The operation is simple and suitable for the purification of soluble histidine tag proteins expressed in the supernatant or cells of bacteria, yeast and cells. it can also be combined with high-throughput protein purification equipment for protein screening and separation.

Co2+ has 4 ligands and less non-specific adsorption, Ni2+ has 6 ligands and strong protein binding ability, for more target proteins, you can choose our other SweMagrose IDA-Ni Magnetic Agarose Beads for His-Tag Protein Purification(G3654).

### Storage and Shipping Conditions

Ship with wet ice; Store at 4°C, valid for 12 months.

### **Product Components**

Component Number	Component	G3655-1ML	G3655-5ML
G3655	SweMagrose IDA-Co Magnetic Agarose Beads for His-Tag Protein Purification	1 mL	5 mL
	Manual	1	C

### Assay Protocol/Procedures

1. The configuration of various reagents can be referred to as follows. Users can choose the appropriate amount of magnetic beads and buffer formula according to the situation of the reagents.

Component	Volume
10×PBS (200 mM Sodium Phosphate, pH7.4)	50 mL
10x Imidazole buffer (200 mM Sodium Phosphate, 5 M Imidazole, pH7.4)	50 mL
Cobalt remover(10 mM Tris-HCl, 500 mM NaCl, 100 mM EDTA, pH7.4)	10 mL
Alkaline washing solution (0.5 M NaOH, 2 M NaCl)	10 mL
Cobaltous sulfate (100 mM CoSO₄)	15 mL
Preservation solution (20% ethanol)	50 mL

### 2. Buffer configuration

The binding performance of the target protein with metal ion chelating beads will directly affect the purification efficiency of the target protein, and various buffers will also affect the recovery and purity of the target protein to some extent. Therefore, before large-scale protein purification, users should design their own experiments to screen the buffers suitable for the target protein, including binding buffer, washing buffer and elution buffer.

The buffer system provided below is suitable for the purification of most histidine tag proteins for

users' reference .:

**Binding Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 5~50 mM Imidazole, pH7.4 **Wash Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 50~100 mM Imidazole, pH7.4 **Elution Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 500 mM Imidazole, pH7.4

### 3. Sample treatment

- a) E. coli, yeast and other intracellularly expressed proteins: add 5~10 mL of Binding Buffer per gram of cells, add protease inhibitor (e.g., PMSF at a final concentration of 1 mM), resuspend the cells, and ultrasonically lysed the cells in an ice bath, i.e., the crude protein sample. If the sample is too viscous, appropriate amount of nuclease can be added to the crude sample as needed and placed on ice for 30 min to degrade the nucleic acids. Alternatively, centrifugation of the protein sample can be performed as needed.
- b) Extracellular expressed protein: The crude protein sample was obtained when the extracellular expression supernatant was diluted with an equal amount of Binding Buffer.
- a) Intracellularly expressed proteins in animal cells: Take appropriate amount of animal cells, wash with appropriate amount of PBS (self-provided) once, discard the supernatant, resuspend with appropriate amount of Binding Buffer containing 1% (v/v) Triton X-100 or 1% (v/v) NP-40, add protease inhibitor (e.g., PMSF at a final concentration of 1 mM), and put it on ice for 10 min, that is crude protein sample.

### 4. Binding of target protein to magnetic beads:

- a) Suspend 2 g wet weight of the organisms with 10 mL of Binding Buffer, and after fragmentation and lysis, a sample of the target crude protein is added to a centrifuge tube containing pre-treated magnetic beads, and the tube is placed in a vortex mixer and shaken for 15 s. The target crude protein sample is then added to a centrifuge tube with pre-treated magnetic beads.
- b) Place the centrifuge tube on a rotary mixer for 20-30 min at room temperature (if needed, can rotate and mix for 1 h at a low temperature of 2-8°C to prevent degradation of the target protein).
- c) Place the centrifuge tube on the magnetic separator for separation, transfer the supernatant to a new centrifuge tube for subsequent testing, and remove the centrifuge tube from the magnetic separator for subsequent washing steps.

### 5. Magnetic bead washing:

- a) Add 10 mL of Wash Buffer to the centrifuge tube containing the magnetic beads, gently turn the tube several times to resuspend the beads, separate them magnetically, and transfer the wash solution to a new centrifuge tube for sampling. Repeat this procedure once.
- b) Add 10 mL of Wash Buffer to the centrifuge tube containing magnetic beads to resuspend the beads, transfer the bead suspension to a new centrifuge tube (to avoid contamination of target proteins by non-specifically adsorbed proteins on the wall of the original centrifuge tube), magnetically separate, and pipette the supernatant into the Wash Buffer Collection Tube.

### 6. Target protein elution

- a) Users can adjust the concentration of target protein by changing the elution volume as needed. Add 2-10 mL of Elution Buffer, gently turn the tube several times to suspend the beads, magnetically separate the beads, and collect the eluate into a new centrifuge tube, which is the purified target protein sample.
- b) If necessary, repeat the above steps once to collect the sample into a new centrifuge tube to test whether the target protein is eluted completely.

c) Detect the target protein by SDS-PAGE or Western blotting. If you need to measure the protein concentration, you can use Elution Buffer to zero the concentration, or use dialysis or ultrafiltration to remove Imidazole and then measure the concentration.

### 7. Magnetic bead post-processing

- a) Add 5 mL of Elution Buffer to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant..
- b) Repeat the above steps twice.
- c) Add 5 mL of ddH2O to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant.
- d) Repeat the above steps twice.
- e) Add the preservation solution to the magnetic beads to make a total volume of 5 mL, store at 2-30°C (for long-term storage, place at 2-8°C), and can be used for the next purification of the same protein.

### 8. Magnetic bead regeneration

When the magnetic beads are used continuously for 3 or more times, the ability to bind target proteins may be significantly reduced, and it is recommended to carry out the magnetic bead regeneration process. Take 5 mL of 10% (v/v) magnetic bead suspension as an example to illustrate the magnetic bead regeneration operation in detail.

- a) The magnetic bead suspension was magnetically separated, remove the supernatant, and remove the centrifuge tube from the magnetic separator, add 5 mL of ddH2O to the centrifuge tube and turn the centrifuge tube up and down several times to re-suspend the magnetic beads, magnetically separated and remove the supernatant.
- b) Add 5 mL of cobalt remover, turn the centrifuge tube up and down several times to resuspend the magnetic beads, rotate and mix for 5 min at room temperature, separate magnetically and remove the supernatant. Repeat this step once.
- c) Add 5 mL of ddH2O to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant.
- d) Alkaline treatment (optional step): add 5 mL of alkaline wash buffer, turn the centrifuge tube up and down several times to re-suspend the beads, rotate and mix for 5 min at room temperature, separate magnetically and remove the supernatant. Add 5 mL of ddH2O, turn the centrifuge tube up and down several times to resuspend the beads, magnetically separate and remove the supernatant. Repeat the ddH2O washing step 3-5 times until the washing solution is neutral..
- e) Add 5 mL of nickel chloride, turn the centrifuge tube up and down several times to re-suspend the beads, rotate and mix for 20 min at room temperature, separate magnetically and remove the supernatant.
- f) Add 5 mL of ddH2O and turn the centrifuge tube up and down several times to resuspend the beads, magnetically separate and remove the supernatant. Repeat this step 4 times.
- g) Add the preservation solution to the magnetic beads to make a total volume of 5 mL and store at 2-30°C (for long-term storage, place at 2-8°C).

### Note

1. This product is stored in 20% ethanol and should be replaced before use.

# Servicebio<sup>®</sup> SweMagrose IDA-Co Magnetic Agarose Beads for His-Tag Protein Purification

### Cat. #:G3655

### **Product Information**

Product Name	Cat. No.	Spec.
SweMagrose IDA-Co Magnetic Agarose Beads for	G3655-1ML	1 mL
His-Tag Protein Purification	G3655-5ML	5 mL

### Product Description/Introduction

This product is prepared from agarose magnetic beads modified by IDA and chelating nickel ions. It can efficiently and quickly purify the histidine-tagged proteins in biological samples without centrifugal filtration. The operation is simple and suitable for the purification of soluble histidine tag proteins expressed in the supernatant or cells of bacteria, yeast and cells. it can also be combined with high-throughput protein purification equipment for protein screening and separation.

Co2+ has 4 ligands and less non-specific adsorption, Ni2+ has 6 ligands and strong protein binding ability, for more target proteins, you can choose our other SweMagrose IDA-Ni Magnetic Agarose Beads for His-Tag Protein Purification(G3654).

### Storage and Shipping Conditions

Ship with wet ice; Store at 4°C, valid for 12 months.

### **Product Components**

Component Number	Component	G3655-1ML	G3655-5ML
G3655	SweMagrose IDA-Co Magnetic Agarose Beads for His-Tag Protein Purification	1 mL	5 mL
	Manual	1	C

### Assay Protocol/Procedures

1. The configuration of various reagents can be referred to as follows. Users can choose the appropriate amount of magnetic beads and buffer formula according to the situation of the reagents.

Component	Volume
10×PBS (200 mM Sodium Phosphate, pH7.4)	50 mL
10x Imidazole buffer (200 mM Sodium Phosphate, 5 M Imidazole, pH7.4)	50 mL
Cobalt remover(10 mM Tris-HCl, 500 mM NaCl, 100 mM EDTA, pH7.4)	10 mL
Alkaline washing solution (0.5 M NaOH, 2 M NaCl)	10 mL
Cobaltous sulfate (100 mM CoSO₄)	15 mL
Preservation solution (20% ethanol)	50 mL

### 2. Buffer configuration

The binding performance of the target protein with metal ion chelating beads will directly affect the purification efficiency of the target protein, and various buffers will also affect the recovery and purity of the target protein to some extent. Therefore, before large-scale protein purification, users should design their own experiments to screen the buffers suitable for the target protein, including binding buffer, washing buffer and elution buffer.

The buffer system provided below is suitable for the purification of most histidine tag proteins for

users' reference .:

**Binding Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 5~50 mM Imidazole, pH7.4 **Wash Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 50~100 mM Imidazole, pH7.4 **Elution Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 500 mM Imidazole, pH7.4

### 3. Sample treatment

- a) E. coli, yeast and other intracellularly expressed proteins: add 5~10 mL of Binding Buffer per gram of cells, add protease inhibitor (e.g., PMSF at a final concentration of 1 mM), resuspend the cells, and ultrasonically lysed the cells in an ice bath, i.e., the crude protein sample. If the sample is too viscous, appropriate amount of nuclease can be added to the crude sample as needed and placed on ice for 30 min to degrade the nucleic acids. Alternatively, centrifugation of the protein sample can be performed as needed.
- b) Extracellular expressed protein: The crude protein sample was obtained when the extracellular expression supernatant was diluted with an equal amount of Binding Buffer.
- a) Intracellularly expressed proteins in animal cells: Take appropriate amount of animal cells, wash with appropriate amount of PBS (self-provided) once, discard the supernatant, resuspend with appropriate amount of Binding Buffer containing 1% (v/v) Triton X-100 or 1% (v/v) NP-40, add protease inhibitor (e.g., PMSF at a final concentration of 1 mM), and put it on ice for 10 min, that is crude protein sample.

### 4. Binding of target protein to magnetic beads:

- a) Suspend 2 g wet weight of the organisms with 10 mL of Binding Buffer, and after fragmentation and lysis, a sample of the target crude protein is added to a centrifuge tube containing pre-treated magnetic beads, and the tube is placed in a vortex mixer and shaken for 15 s. The target crude protein sample is then added to a centrifuge tube with pre-treated magnetic beads.
- b) Place the centrifuge tube on a rotary mixer for 20-30 min at room temperature (if needed, can rotate and mix for 1 h at a low temperature of 2-8°C to prevent degradation of the target protein).
- c) Place the centrifuge tube on the magnetic separator for separation, transfer the supernatant to a new centrifuge tube for subsequent testing, and remove the centrifuge tube from the magnetic separator for subsequent washing steps.

### 5. Magnetic bead washing:

- a) Add 10 mL of Wash Buffer to the centrifuge tube containing the magnetic beads, gently turn the tube several times to resuspend the beads, separate them magnetically, and transfer the wash solution to a new centrifuge tube for sampling. Repeat this procedure once.
- b) Add 10 mL of Wash Buffer to the centrifuge tube containing magnetic beads to resuspend the beads, transfer the bead suspension to a new centrifuge tube (to avoid contamination of target proteins by non-specifically adsorbed proteins on the wall of the original centrifuge tube), magnetically separate, and pipette the supernatant into the Wash Buffer Collection Tube.

### 6. Target protein elution

- a) Users can adjust the concentration of target protein by changing the elution volume as needed. Add 2-10 mL of Elution Buffer, gently turn the tube several times to suspend the beads, magnetically separate the beads, and collect the eluate into a new centrifuge tube, which is the purified target protein sample.
- b) If necessary, repeat the above steps once to collect the sample into a new centrifuge tube to test whether the target protein is eluted completely.

c) Detect the target protein by SDS-PAGE or Western blotting. If you need to measure the protein concentration, you can use Elution Buffer to zero the concentration, or use dialysis or ultrafiltration to remove Imidazole and then measure the concentration.

### 7. Magnetic bead post-processing

- a) Add 5 mL of Elution Buffer to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant..
- b) Repeat the above steps twice.
- c) Add 5 mL of ddH2O to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant.
- d) Repeat the above steps twice.
- e) Add the preservation solution to the magnetic beads to make a total volume of 5 mL, store at 2-30°C (for long-term storage, place at 2-8°C), and can be used for the next purification of the same protein.

### 8. Magnetic bead regeneration

When the magnetic beads are used continuously for 3 or more times, the ability to bind target proteins may be significantly reduced, and it is recommended to carry out the magnetic bead regeneration process. Take 5 mL of 10% (v/v) magnetic bead suspension as an example to illustrate the magnetic bead regeneration operation in detail.

- a) The magnetic bead suspension was magnetically separated, remove the supernatant, and remove the centrifuge tube from the magnetic separator, add 5 mL of ddH2O to the centrifuge tube and turn the centrifuge tube up and down several times to re-suspend the magnetic beads, magnetically separated and remove the supernatant.
- b) Add 5 mL of cobalt remover, turn the centrifuge tube up and down several times to resuspend the magnetic beads, rotate and mix for 5 min at room temperature, separate magnetically and remove the supernatant. Repeat this step once.
- c) Add 5 mL of ddH2O to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant.
- d) Alkaline treatment (optional step): add 5 mL of alkaline wash buffer, turn the centrifuge tube up and down several times to re-suspend the beads, rotate and mix for 5 min at room temperature, separate magnetically and remove the supernatant. Add 5 mL of ddH2O, turn the centrifuge tube up and down several times to resuspend the beads, magnetically separate and remove the supernatant. Repeat the ddH2O washing step 3-5 times until the washing solution is neutral..
- e) Add 5 mL of nickel chloride, turn the centrifuge tube up and down several times to re-suspend the beads, rotate and mix for 20 min at room temperature, separate magnetically and remove the supernatant.
- f) Add 5 mL of ddH2O and turn the centrifuge tube up and down several times to resuspend the beads, magnetically separate and remove the supernatant. Repeat this step 4 times.
- g) Add the preservation solution to the magnetic beads to make a total volume of 5 mL and store at 2-30°C (for long-term storage, place at 2-8°C).

### Note

1. This product is stored in 20% ethanol and should be replaced before use.

# Servicebio<sup>®</sup> SweMagrose IDA-Ni Magnetic Agarose Beads for His-Tag Protein Purification

### Cat. #:G3654

### **Product Information**

Product Name	Cat. No.	Spec.
SweMagrose IDA-Ni Magnetic Agarose Beads for	G3654-1ML	1 mL
His-Tag Protein Purification	G3654-5ML	5 mL

### Product Description/Introduction

This product is prepared from agarose magnetic beads modified by IDA and chelating nickel ions. It can efficiently and quickly purify the histidine-tagged proteins in biological samples without centrifugal filtration. The operation is simple and suitable for the purification of soluble histidine tag proteins expressed in the supernatant or cells of bacteria, yeast and cells. it can also be combined with high-throughput protein purification equipment for protein screening and separation.

Ni<sup>2+</sup> has 6 ligands and strong protein binding ability, Co<sup>2+</sup> has 4 ligands and less non-specific adsorption, for higher purity requirements, you can choose our other SweMagrose IDA-Co Magnetic Agarose Beads for His-Tag Protein Purification(G3655).

### Storage and Shipping Conditions

Ship with wet ice; Store at 4°C, valid for 12 months.

### **Product Components**

Component Number	Component	G3654-1ML	G3654-5ML
G3654	SweMagrose IDA-Ni Magnetic Agarose Beads for His-Tag Protein Purification	1 mL	5 mL
	Manual	1	C

### Assay Protocol/Procedures

1. The configuration of various reagents can be referred to as follows. Users can choose the appropriate amount of magnetic beads and buffer formula according to the situation of the reagents.

Component	Volume
10×PBS (200 mM Sodium Phosphate, pH7.4)	50 mL
10x Imidazole buffer(200 mM Sodium Phosphate, 5 M Imidazole, pH7.4)	50 mL
Nickel remover(10 mM Tris-HCl, 500 mM NaCl, 100 mM EDTA, pH7.4)	10 mL
Alkaline washing solution (0.5 M NaOH, 2M NaCl)	10 mL
Nickel chloride (100 mM NiCl <sub>2</sub> )	15 mL
Preservation solution (20% ethanol)	50 mL

### 2. Buffer configuration

- a) The binding performance of the target protein with metal ion chelating beads will directly affect the purification efficiency of the target protein, and various buffers will also affect the recovery and purity of the target protein to some extent. Therefore, before large-scale protein purification, users should design their own experiments to screen the buffers suitable for the target protein, including binding buffer, washing buffer and elution buffer.
- b) The buffer system provided below is suitable for the purification of most histidine tag proteins for

users' reference.

**Binding Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 5~50 mM Imidazole, pH7.4 **Wash Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 50~100 mM Imidazole, pH7.4 **Elution Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 500 mM Imidazole, pH7.4

### 3. Sample treatment

- a) E. coli, yeast and other intracellularly expressed proteins: add 5~10 mL of Binding Buffer per gram of cells, add protease inhibitor (e.g., PMSF at a final concentration of 1 mM), resuspend the cells, and ultrasonically lysed the cells in an ice bath, i.e., the crude protein sample. If the sample is too viscous, appropriate amount of nuclease can be added to the crude sample as needed and placed on ice for 30 min to degrade the nucleic acids. Alternatively, centrifugation of the protein sample can be performed as needed.
- b) Extracellular expressed protein: The crude protein sample was obtained when the extracellular expression supernatant was diluted with an equal amount of Binding Buffer.
- c) Intracellularly expressed proteins in animal cells: Take appropriate amount of animal cells, wash with appropriate amount of PBS (self-provided) once, discard the supernatant, resuspend with appropriate amount of Binding Buffer containing 1% (v/v) Triton X-100 or 1% (v/v) NP-40, add protease inhibitor (e.g., PMSF at a final concentration of 1 mM), and put it on ice for 10 min, that is crude protein sample.

### 4. Binding of target protein to magnetic beads:

- a) Suspend 2 g wet weight of the organisms with 10 mL of Binding Buffer, and after fragmentation and lysis, a sample of the target crude protein is added to a centrifuge tube containing pre-treated magnetic beads, and the tube is placed in a vortex mixer and shaken for 15 s. The target crude protein sample is then added to a centrifuge tube with pre-treated magnetic beads.
- b) Place the centrifuge tube on a rotary mixer for 20-30 min at room temperature (if needed, can rotate and mix for 1 h at a low temperature of 2-8°C to prevent degradation of the target protein).
- c) Place the centrifuge tube on the magnetic separator for separation, transfer the supernatant to a new centrifuge tube for subsequent testing, and remove the centrifuge tube from the magnetic separator for subsequent washing steps.

### 5. Magnetic bead washing:

- a) Add 10 mL of Wash Buffer to the centrifuge tube containing the magnetic beads, gently turn the tube several times to resuspend the beads, separate them magnetically, and transfer the wash solution to a new centrifuge tube for sampling. Repeat this procedure once.
- b) Add 10 mL of Wash Buffer to the centrifuge tube containing magnetic beads to resuspend the beads, transfer the bead suspension to a new centrifuge tube (to avoid contamination of target proteins by non-specifically adsorbed proteins on the wall of the original centrifuge tube), magnetically separate, and pipette the supernatant into the Wash Buffer Collection Tube.

### 6. Target protein elution

- a) Users can adjust the concentration of target protein by changing the elution volume as needed. Add 2-10 mL of Elution Buffer, gently turn the tube several times to suspend the beads, magnetically separate the beads, and collect the eluate into a new centrifuge tube, which is the purified target protein sample.
- b) If necessary, repeat the above steps once to collect the sample into a new centrifuge tube to test whether the target protein is eluted completely.
c) Detect the target protein by SDS-PAGE or Western blotting. If you need to measure the protein concentration, you can use Elution Buffer to zero the concentration, or use dialysis or ultrafiltration to remove Imidazole and then measure the concentration.

#### 7. Magnetic bead post-processing

- a) Add 5 mL of Elution Buffer to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant..
- b) Repeat the above steps twice.
- c) Add 5 mL of ddH2O to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant.
- d) Repeat the above steps twice.
- e) Add the preservation solution to the magnetic beads to make a total volume of 5 mL, store at 2-30°C (for long-term storage, place at 2-8°C), and can be used for the next purification of the same protein.

## 8. Magnetic bead regeneration

When the magnetic beads are used continuously for 3 or more times, the ability to bind target proteins may be significantly reduced, and it is recommended to carry out the magnetic bead regeneration process. Take 5 mL of 10% (v/v) magnetic bead suspension as an example to illustrate the magnetic bead regeneration operation in detail.

- a) The magnetic bead suspension was magnetically separated, remove the supernatant, and remove the centrifuge tube from the magnetic separator, add 5 mL of ddH2O to the centrifuge tube and turn the centrifuge tube up and down several times to re-suspend the magnetic beads, magnetically separated and remove the supernatant.
- b) Add 5 mL of nickel remover, turn the centrifuge tube up and down several times to resuspend the magnetic beads, rotate and mix for 5 min at room temperature, separate magnetically and remove the supernatant. Repeat this step once.
- c) Add 5 mL of ddH2O to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant.
- d) Alkaline treatment (optional step): add 5 mL of alkaline wash buffer, turn the centrifuge tube up and down several times to re-suspend the beads, rotate and mix for 5 min at room temperature, separate magnetically and remove the supernatant. Add 5 mL of ddH2O, turn the centrifuge tube up and down several times to resuspend the beads, magnetically separate and remove the supernatant. Repeat the ddH2O washing step 3-5 times until the washing solution is neutral..
- e) Add 5 mL of nickel chloride, turn the centrifuge tube up and down several times to re-suspend the beads, rotate and mix for 20 min at room temperature, separate magnetically and remove the supernatant.
- f) Add 5 mL of ddH2O and turn the centrifuge tube up and down several times to resuspend the beads, magnetically separate and remove the supernatant. Repeat this step 4 times.
- g) Add the preservation solution to the magnetic beads to make a total volume of 5 mL and store at 2-30°C (for long-term storage, place at 2-8°C).

## Note

1. This product is stored in 20% ethanol and should be replaced before use.

# Servicebio<sup>®</sup> SweMagrose IDA-Ni Magnetic Agarose Beads for His-Tag Protein Purification

## Cat. #:G3654

## **Product Information**

Product Name	Cat. No.	Spec.
SweMagrose IDA-Ni Magnetic Agarose Beads for	G3654-1ML	1 mL
His-Tag Protein Purification	G3654-5ML	5 mL

## Product Description/Introduction

This product is prepared from agarose magnetic beads modified by IDA and chelating nickel ions. It can efficiently and quickly purify the histidine-tagged proteins in biological samples without centrifugal filtration. The operation is simple and suitable for the purification of soluble histidine tag proteins expressed in the supernatant or cells of bacteria, yeast and cells. it can also be combined with high-throughput protein purification equipment for protein screening and separation.

Ni<sup>2+</sup> has 6 ligands and strong protein binding ability, Co<sup>2+</sup> has 4 ligands and less non-specific adsorption, for higher purity requirements, you can choose our other SweMagrose IDA-Co Magnetic Agarose Beads for His-Tag Protein Purification(G3655).

## Storage and Shipping Conditions

Ship with wet ice; Store at 4°C, valid for 12 months.

## **Product Components**

Component Number	Component	G3654-1ML	G3654-5ML
G3654	SweMagrose IDA-Ni Magnetic Agarose Beads for His-Tag Protein Purification	1 mL	5 mL
	Manual	1	C

## Assay Protocol/Procedures

1. The configuration of various reagents can be referred to as follows. Users can choose the appropriate amount of magnetic beads and buffer formula according to the situation of the reagents.

Component	Volume
10×PBS (200 mM Sodium Phosphate, pH7.4)	50 mL
10x Imidazole buffer(200 mM Sodium Phosphate, 5 M Imidazole, pH7.4)	50 mL
Nickel remover(10 mM Tris-HCl, 500 mM NaCl, 100 mM EDTA, pH7.4)	10 mL
Alkaline washing solution (0.5 M NaOH, 2M NaCl)	10 mL
Nickel chloride (100 mM NiCl <sub>2</sub> )	15 mL
Preservation solution (20% ethanol)	50 mL

## 2. Buffer configuration

- a) The binding performance of the target protein with metal ion chelating beads will directly affect the purification efficiency of the target protein, and various buffers will also affect the recovery and purity of the target protein to some extent. Therefore, before large-scale protein purification, users should design their own experiments to screen the buffers suitable for the target protein, including binding buffer, washing buffer and elution buffer.
- b) The buffer system provided below is suitable for the purification of most histidine tag proteins for

users' reference.

**Binding Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 5~50 mM Imidazole, pH7.4 **Wash Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 50~100 mM Imidazole, pH7.4 **Elution Buffer:** 20 mM Sodium Phosphate, 500 mM NaCl, 500 mM Imidazole, pH7.4

## 3. Sample treatment

- a) E. coli, yeast and other intracellularly expressed proteins: add 5~10 mL of Binding Buffer per gram of cells, add protease inhibitor (e.g., PMSF at a final concentration of 1 mM), resuspend the cells, and ultrasonically lysed the cells in an ice bath, i.e., the crude protein sample. If the sample is too viscous, appropriate amount of nuclease can be added to the crude sample as needed and placed on ice for 30 min to degrade the nucleic acids. Alternatively, centrifugation of the protein sample can be performed as needed.
- b) Extracellular expressed protein: The crude protein sample was obtained when the extracellular expression supernatant was diluted with an equal amount of Binding Buffer.
- c) Intracellularly expressed proteins in animal cells: Take appropriate amount of animal cells, wash with appropriate amount of PBS (self-provided) once, discard the supernatant, resuspend with appropriate amount of Binding Buffer containing 1% (v/v) Triton X-100 or 1% (v/v) NP-40, add protease inhibitor (e.g., PMSF at a final concentration of 1 mM), and put it on ice for 10 min, that is crude protein sample.

#### 4. Binding of target protein to magnetic beads:

- a) Suspend 2 g wet weight of the organisms with 10 mL of Binding Buffer, and after fragmentation and lysis, a sample of the target crude protein is added to a centrifuge tube containing pre-treated magnetic beads, and the tube is placed in a vortex mixer and shaken for 15 s. The target crude protein sample is then added to a centrifuge tube with pre-treated magnetic beads.
- b) Place the centrifuge tube on a rotary mixer for 20-30 min at room temperature (if needed, can rotate and mix for 1 h at a low temperature of 2-8°C to prevent degradation of the target protein).
- c) Place the centrifuge tube on the magnetic separator for separation, transfer the supernatant to a new centrifuge tube for subsequent testing, and remove the centrifuge tube from the magnetic separator for subsequent washing steps.

#### 5. Magnetic bead washing:

- a) Add 10 mL of Wash Buffer to the centrifuge tube containing the magnetic beads, gently turn the tube several times to resuspend the beads, separate them magnetically, and transfer the wash solution to a new centrifuge tube for sampling. Repeat this procedure once.
- b) Add 10 mL of Wash Buffer to the centrifuge tube containing magnetic beads to resuspend the beads, transfer the bead suspension to a new centrifuge tube (to avoid contamination of target proteins by non-specifically adsorbed proteins on the wall of the original centrifuge tube), magnetically separate, and pipette the supernatant into the Wash Buffer Collection Tube.

## 6. Target protein elution

- a) Users can adjust the concentration of target protein by changing the elution volume as needed. Add 2-10 mL of Elution Buffer, gently turn the tube several times to suspend the beads, magnetically separate the beads, and collect the eluate into a new centrifuge tube, which is the purified target protein sample.
- b) If necessary, repeat the above steps once to collect the sample into a new centrifuge tube to test whether the target protein is eluted completely.

c) Detect the target protein by SDS-PAGE or Western blotting. If you need to measure the protein concentration, you can use Elution Buffer to zero the concentration, or use dialysis or ultrafiltration to remove Imidazole and then measure the concentration.

#### 7. Magnetic bead post-processing

- a) Add 5 mL of Elution Buffer to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant..
- b) Repeat the above steps twice.
- c) Add 5 mL of ddH2O to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant.
- d) Repeat the above steps twice.
- e) Add the preservation solution to the magnetic beads to make a total volume of 5 mL, store at 2-30°C (for long-term storage, place at 2-8°C), and can be used for the next purification of the same protein.

## 8. Magnetic bead regeneration

When the magnetic beads are used continuously for 3 or more times, the ability to bind target proteins may be significantly reduced, and it is recommended to carry out the magnetic bead regeneration process. Take 5 mL of 10% (v/v) magnetic bead suspension as an example to illustrate the magnetic bead regeneration operation in detail.

- a) The magnetic bead suspension was magnetically separated, remove the supernatant, and remove the centrifuge tube from the magnetic separator, add 5 mL of ddH2O to the centrifuge tube and turn the centrifuge tube up and down several times to re-suspend the magnetic beads, magnetically separated and remove the supernatant.
- b) Add 5 mL of nickel remover, turn the centrifuge tube up and down several times to resuspend the magnetic beads, rotate and mix for 5 min at room temperature, separate magnetically and remove the supernatant. Repeat this step once.
- c) Add 5 mL of ddH2O to the centrifuge tube containing the magnetic beads, turn the tube up and down several times to suspend the beads, separate them magnetically and remove the supernatant.
- d) Alkaline treatment (optional step): add 5 mL of alkaline wash buffer, turn the centrifuge tube up and down several times to re-suspend the beads, rotate and mix for 5 min at room temperature, separate magnetically and remove the supernatant. Add 5 mL of ddH2O, turn the centrifuge tube up and down several times to resuspend the beads, magnetically separate and remove the supernatant. Repeat the ddH2O washing step 3-5 times until the washing solution is neutral..
- e) Add 5 mL of nickel chloride, turn the centrifuge tube up and down several times to re-suspend the beads, rotate and mix for 20 min at room temperature, separate magnetically and remove the supernatant.
- f) Add 5 mL of ddH2O and turn the centrifuge tube up and down several times to resuspend the beads, magnetically separate and remove the supernatant. Repeat this step 4 times.
- g) Add the preservation solution to the magnetic beads to make a total volume of 5 mL and store at 2-30°C (for long-term storage, place at 2-8°C).

## Note

1. This product is stored in 20% ethanol and should be replaced before use.

# Servicebio<sup>®</sup> SweMagrose COOH

## Cat. #: G3652

## **Product Information**

Product Name	Cat. No.	Spec.
	G3652-1ML	1 mL
Sweivlagrose COOH	G3652-5ML	5 mL

## **Product Description/Introduction**

Magnetic separation technology can easily separate or enrich antibodies, antigens, lectins, proteases, nucleic acids and cells while maintaining their biological activity. Magnetic agarose carboxylated beads (SweMagrose COOH) use polymer polymerisation technology to blend superparamagnetic materials and polymers together to make a suspension of superparamagnetic iron oxide microspheres with carboxylate groups on the surface; SweMagrose COOH has faster magnetic responsiveness, good dispersion, extremely low non-specific adsorption, and richer binding sites than traditional magnetic beads. Compared with traditional magnetic beads, SweMagrose COOH has faster magnetic responsiveness, good dispersion, extremely low non-specific adsorption and richer binding sites; SweMagrose COOH can efficiently bind with a variety of biological ligands (oligonucleotides, peptides, proteins, drug molecules, etc.) at high loads with special reagents (e.g., EDC), which is the best way to carry out the subsequent treatments such as encapsulation and adsorption, lt is a good base material for subsequent processing such as encapsulation, adsorption, chemical modification, etc.

Abundant binding sites: Enhances specific binding to ligand

High Magnetic Response: Faster magnetic response saves experiment time.

Superparamagnetic: good dispersion even after the disappearance of the external magnetic field.

**Excellent dispersibility and resuspension:** good dispersibility and resuspension ensure the stability of the experiment and facilitate the operation.

Excellent physicochemical stability: better guarantee of experimental reproducibility.

## **Product Information**

Component	SweMagrose COOH	
Bead Diameter	30~150 μm	
Surface amino content	60 μmol/mL gel	
Preservation solution	20% ethanol solution	
Magnetic core	Fe <sub>3</sub> O <sub>4</sub>	
Shell layer	Agarose	
Magnetization	Superparamagnetism	

## Storage and Shipping Conditions

Ship at room temperature; Store at 2-8°C, valid for 24 months.

#### **Product Components**

Component Number	Component	G3562-1ML	G3562-5ML
G3562	SweMagrose COOH	1 mL	5 mL
Manual		1 p	C

## Assay Protocol/Procedures(Take coupling protein G as an example)

## A. Self-provided reagent preparation

**MEST Solution:** MES (2-morpholine ethanesulfonic acid) 100 mM, 0.05% Tween 20, adjust pH to 5.0 with sodium hydroxide.

EDC (carbodiimide) solution: EDC 10 mg/mL dissolved in MEST solution.

NHS (N-hydroxysuccinimide) solution: NHS 10 mg/mL dissolved in MEST solution.

- B. Carboxyl group activation on magnetic bead surface
  - a) After mixing the agarose carboxylated magnetic beads (SweMagrose COOH), take 100  $\mu$ L into a 1.5 mL EP tube and place it on a magnetic separation rack for 30 s. Remove the supernatant, wash it twice with 200  $\mu$ L of MEST solution by magnetic separation, and then aspirate the supernatant.
  - b) Rapidly add 100 µL of freshly prepared EDC solution and 100 µL of NHS solution to the centrifuge tube containing the beads, vortex to fully suspend the beads, activate for 30 min at 25° C, keep the beads in suspension during this period (vertical mixer can be used for inverted mixing); after the above steps the carboxyl groups on the surface of the beads are activated and ready for covalent coupling with biological ligands with primary amino groups (the activated state is not suitable for prolonged storage, immediate coupling is recommended).

#### C. Covalent coupling of magnetic beads to biological ligands

- a) The supernatant was removed by magnetic suction, add 50~200 µg of biological ligand (dosage, concentration and type of buffer need to be optimised according to the specific experiments, and the following ligand buffers can be used: 100 mM 2-morpholine ethanesulfonic acid buffer, pH 4.8; 200 mM sodium bicarbonate buffer, pH 8.3; 50 mM phosphate buffer, pH 8.5; 100 mM sodium chloride solution, pH 7.4, etc.; 0.05% Tween 20 may be added to the buffer to improve the dispersion of the beads and to avoid the presence of reagents containing primary amines other than biological ligands in the buffer system), mix gently.
- b) Couple at 25 °C for 2 h or 25 °C for 1 h and leave overnight at 4 °C, keeping the beads in suspension during coupling (vertical mixer can be used for inverted mixing)
- c) The centrifuge tube is placed in a magnetic separation rack and the supernatant is removed by magnetic suction, the beads are resuspended (sonicated if required) in 200 μL of PBST solution (pH 7.2 with 1% BSA) and reacted at 25°C for 1 h to seal off unreacted activated carboxyl groups on the surface of the beads, which are kept in suspension during this time (inverted mixing can be carried out using a vertical mixer).
- d) Centrifuge tubes were placed in a magnetic separation rack and the supernatant was removed by magnetic suction separation, washed three times and each time with 200 µL of PBS solution (pH 7.2) or preservation solution, and then re-suspended in preservation solution (the amount of preservation solution added can be determined as needed to adjust the concentration of coupled ligand beads) and stored at 4°C; if the immobilised biologic ligands are stable, 0.02% (w/v) sodium azide (NaN3) can be added to preservation solution as a bacteriostatic inhibitor.

- 1. Operations such as freezing, drying and centrifugation cause agglomeration of the beads, which makes them difficult to resuspend and disperse and affects the chemical activity of the functional groups on the surface of the beads.
- 2. The product is stored in 20% ethanol. Wash the beads 2-3 times with pure water or buffer to remove ethanol from the preservation solution before use.
  - 3. Be sure to shake or sonicate the beads well before using the product so that they are evenly suspended.
  - 4. Use with a matching magnetic holder.

# Servicebio<sup>®</sup> SweMagrose COOH

## Cat. #: G3652

## **Product Information**

Product Name	Cat. No.	Spec.
	G3652-1ML	1 mL
Sweivlagrose COOH	G3652-5ML	5 mL

## **Product Description/Introduction**

Magnetic separation technology can easily separate or enrich antibodies, antigens, lectins, proteases, nucleic acids and cells while maintaining their biological activity. Magnetic agarose carboxylated beads (SweMagrose COOH) use polymer polymerisation technology to blend superparamagnetic materials and polymers together to make a suspension of superparamagnetic iron oxide microspheres with carboxylate groups on the surface; SweMagrose COOH has faster magnetic responsiveness, good dispersion, extremely low non-specific adsorption, and richer binding sites than traditional magnetic beads. Compared with traditional magnetic beads, SweMagrose COOH has faster magnetic responsiveness, good dispersion, extremely low non-specific adsorption and richer binding sites; SweMagrose COOH can efficiently bind with a variety of biological ligands (oligonucleotides, peptides, proteins, drug molecules, etc.) at high loads with special reagents (e.g., EDC), which is the best way to carry out the subsequent treatments such as encapsulation and adsorption, lt is a good base material for subsequent processing such as encapsulation, adsorption, chemical modification, etc.

Abundant binding sites: Enhances specific binding to ligand

High Magnetic Response: Faster magnetic response saves experiment time.

Superparamagnetic: good dispersion even after the disappearance of the external magnetic field.

**Excellent dispersibility and resuspension:** good dispersibility and resuspension ensure the stability of the experiment and facilitate the operation.

Excellent physicochemical stability: better guarantee of experimental reproducibility.

## **Product Information**

Component	SweMagrose COOH	
Bead Diameter	30~150 μm	
Surface amino content	60 μmol/mL gel	
Preservation solution	20% ethanol solution	
Magnetic core	Fe <sub>3</sub> O <sub>4</sub>	
Shell layer	Agarose	
Magnetization	Superparamagnetism	

## Storage and Shipping Conditions

Ship at room temperature; Store at 2-8°C, valid for 24 months.

#### **Product Components**

Component Number	Component	G3562-1ML	G3562-5ML
G3562	SweMagrose COOH	1 mL	5 mL
Manual		1 p	C

## Assay Protocol/Procedures(Take coupling protein G as an example)

## A. Self-provided reagent preparation

**MEST Solution:** MES (2-morpholine ethanesulfonic acid) 100 mM, 0.05% Tween 20, adjust pH to 5.0 with sodium hydroxide.

EDC (carbodiimide) solution: EDC 10 mg/mL dissolved in MEST solution.

NHS (N-hydroxysuccinimide) solution: NHS 10 mg/mL dissolved in MEST solution.

- B. Carboxyl group activation on magnetic bead surface
  - a) After mixing the agarose carboxylated magnetic beads (SweMagrose COOH), take 100  $\mu$ L into a 1.5 mL EP tube and place it on a magnetic separation rack for 30 s. Remove the supernatant, wash it twice with 200  $\mu$ L of MEST solution by magnetic separation, and then aspirate the supernatant.
  - b) Rapidly add 100 µL of freshly prepared EDC solution and 100 µL of NHS solution to the centrifuge tube containing the beads, vortex to fully suspend the beads, activate for 30 min at 25° C, keep the beads in suspension during this period (vertical mixer can be used for inverted mixing); after the above steps the carboxyl groups on the surface of the beads are activated and ready for covalent coupling with biological ligands with primary amino groups (the activated state is not suitable for prolonged storage, immediate coupling is recommended).

#### C. Covalent coupling of magnetic beads to biological ligands

- a) The supernatant was removed by magnetic suction, add 50~200 µg of biological ligand (dosage, concentration and type of buffer need to be optimised according to the specific experiments, and the following ligand buffers can be used: 100 mM 2-morpholine ethanesulfonic acid buffer, pH 4.8; 200 mM sodium bicarbonate buffer, pH 8.3; 50 mM phosphate buffer, pH 8.5; 100 mM sodium chloride solution, pH 7.4, etc.; 0.05% Tween 20 may be added to the buffer to improve the dispersion of the beads and to avoid the presence of reagents containing primary amines other than biological ligands in the buffer system), mix gently.
- b) Couple at 25 °C for 2 h or 25 °C for 1 h and leave overnight at 4 °C, keeping the beads in suspension during coupling (vertical mixer can be used for inverted mixing)
- c) The centrifuge tube is placed in a magnetic separation rack and the supernatant is removed by magnetic suction, the beads are resuspended (sonicated if required) in 200 μL of PBST solution (pH 7.2 with 1% BSA) and reacted at 25°C for 1 h to seal off unreacted activated carboxyl groups on the surface of the beads, which are kept in suspension during this time (inverted mixing can be carried out using a vertical mixer).
- d) Centrifuge tubes were placed in a magnetic separation rack and the supernatant was removed by magnetic suction separation, washed three times and each time with 200 µL of PBS solution (pH 7.2) or preservation solution, and then re-suspended in preservation solution (the amount of preservation solution added can be determined as needed to adjust the concentration of coupled ligand beads) and stored at 4°C; if the immobilised biologic ligands are stable, 0.02% (w/v) sodium azide (NaN3) can be added to preservation solution as a bacteriostatic inhibitor.

- 1. Operations such as freezing, drying and centrifugation cause agglomeration of the beads, which makes them difficult to resuspend and disperse and affects the chemical activity of the functional groups on the surface of the beads.
- 2. The product is stored in 20% ethanol. Wash the beads 2-3 times with pure water or buffer to remove ethanol from the preservation solution before use.
- 3. Be sure to shake or sonicate the beads well before using the product so that they are evenly suspended.
- 4. Use with a matching magnetic holder.

# Servicebio® SweMagrose OH

## Cat. #: G3651

## **Product Information**

Product Name	Cat. No.	Spec.
SweMagross OH	G3651-1ML	1 mL
Sweivlagrose OH	G3651-5ML	5 mL

## Product Description/Introduction

This product is an agarose microsphere embedded with superparamagnetic  $Fe_3O_4$ , rich in hydroxyl groups on the surface, with very low non-specific adsorption and rich binding sites, capable of efficiently coupling antibodies, peptides, oligonucleotides, organic molecules, etc., under the action of special chemical reagents, such as epichlorohydrin and CDI, and it can be used as an ideal raw material for the research of interdisciplinary disciplines, such as medical science, biology and chemistry.

## **Storage and Shipping Conditions**

Ambient shipping. Store at 2-8°C, valid for 24 months.

## **Product Components**

Component Number	Component	G3651-1ML	G3651-5ML
G3651	SweMagrose OH	1 mL	5 mL
Manual		1	рс

## **Product Applications**

- 1. Fixed antibodies, peptides, proteins, oligonucleotides, organic molecules, etc.
- 2. Protein purification, protein interactions, protein-nucleic acid interactions and other studies

- 1. This product is stored in 20% ethanol and should be replaced before use.
- 2. Do not freeze or dry the beads, as this may affect the final use.
- 3. Use with a matching magnetic frame.

# Servicebio® SweMagrose OH

## Cat. #: G3651

## **Product Information**

Product Name	Cat. No.	Spec.
SweMagross OH	G3651-1ML	1 mL
Sweivlagrose OH	G3651-5ML	5 mL

## Product Description/Introduction

This product is an agarose microsphere embedded with superparamagnetic  $Fe_3O_4$ , rich in hydroxyl groups on the surface, with very low non-specific adsorption and rich binding sites, capable of efficiently coupling antibodies, peptides, oligonucleotides, organic molecules, etc., under the action of special chemical reagents, such as epichlorohydrin and CDI, and it can be used as an ideal raw material for the research of interdisciplinary disciplines, such as medical science, biology and chemistry.

## **Storage and Shipping Conditions**

Ambient shipping. Store at 2-8°C, valid for 24 months.

## **Product Components**

Component Number	Component	G3651-1ML	G3651-5ML
G3651	SweMagrose OH	1 mL	5 mL
Manual		1	рс

## **Product Applications**

- 1. Fixed antibodies, peptides, proteins, oligonucleotides, organic molecules, etc.
- 2. Protein purification, protein interactions, protein-nucleic acid interactions and other studies

- 1. This product is stored in 20% ethanol and should be replaced before use.
- 2. Do not freeze or dry the beads, as this may affect the final use.
- 3. Use with a matching magnetic frame.



# Servicebio<sup>®</sup> SweAgrose Protein g Antibody Purified Agarose

## Cat. #: G2207

## **Product Information**

Product Name	Cat. No.	Spec.
Sucharasa Dratain C. Antihady Durifiad agarasa	G2207-1ML	1 mL
SweAgrose Protein & Antibody Purned agarose	G2207-5ML	5 mL

## Product Description/Introduction

Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42 kDa. Protein G is an immunoglobulin-binding protein expressed by Streptococcal bacteria (C or G). Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to agarose can be used for immunoprecipitation or antibody purification. Protein A agarose is suitable for binding human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, while Protein G agarose is suitable for binding human IgG1, IgG2, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c and other polyclonal antibodies. (See Table I for specific information) This product adopts the self-developed and produced Protein G labelled agarose, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc..

Characteristics	Description
Product content	50% (v/v) agarose in specific protective buffer
Beads structure	6% cross-linked agarose
Coupled protein	Protein G
M.W.of protein	~25 kDa(Protein G)
Binding capacity	>1mg mouse antibody per ml beads
Specificity	Antibodies from different species, including mice, humans, rats, goats, sheep and cattle
Beads size	30~150 μm
Elution method	Elution with acid, or 1x SDS-PAGE loading buffer (reduced) Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~ 50kDa) and light chain (~ 25kDa) of the antibody will be denatured and released from the agarose beads.
Application	IP, Co-IP, Protein purification

#### **Product Information**

## **Storage and Shipping Conditions**

Ship with wet ice; Store at 2-8°C, valid for 12 months.

#### **Product Components**

Component Number	Component	G2207-1ML	G2207-5ML
G2207	SweAgrose Protein G Antibody Purified agarose	1 mL	5 mL
Manual		1p	C

## **Experiment preparation**

Antibody purification related reagent formulations refer to the following, the user can be adjusted according to specific experimental conditions.

Component	Reagent combination		
Pinding Wash Puffer	PBST: 137 mM NaCl, 2.7 mM KCl, 10 mM Na <sub>2</sub> HPO <sub>4</sub> ,		
Binding wash Butter	2.0 mM KH <sub>2</sub> PO <sub>4</sub> , 0.1% Tween-20		
Elution Buffer	100 mM Gly, 0.1% Tween-20, pH 2.5		
Neutralization Buffer	1.0 M Tris-HCl, pH 9.0		
Preservation Buffer	PBST, 0.1% (v/v) Proclin 300		

## Manual procedure (purification of mouse ascites IgG as an example)

- 1. **Sample processing:** take 500 μL of ascites sample, add the binding wash buffer to make up 500 μL, if there are more protein precipitates in the sample, centrifuge the supernatant for experiments, which can improve the purity of the antibody.
- 2. Agarose pretreatment: Vortex the antibody purified agarose for 30 s to resuspend sufficiently, take 100 μL of 50% (v/v) SweAgarose Protein G Antibody Purified agarose in another new 1.5 mL EP tube, centrifuge at 6,000 x g for 30 s at 4 °C and discard the supernatant, wash it with 1 mL of binding washing buffer for twice, and then take the supernatant after magnetically aspirating.

Note: The amount of agarose can be adjusted according to the amount of antibody in the sample.

- 3. Antibody adsorption: add the sample processed in step 1 to the agarose in step 2, vortex and mix well, place the EP tube in a rotary mixer or manually turn the tube gently at room temperature (about 25°C) to make the agarose full contact with the sample, turn it over for 15 min, centrifuge at 6,000 x g for 30 s at 4°C, and then discard the supernatant.
- 4. **Agarose washing:** add 1 mL of binding wash buffer to the EP tube, resuspend with shaking and then centrifuge at 6,000 x g for 30 s at 4°C. Discard the supernatant and repeat the operation 3 times.
- 5. Antibody elution: Add 0.5~1.0 mL of eluent to the EP tubes with the agarose washed as described above, and resuspend the tubes rapidly by pipetting or vortexing, and then gently turn the tubes over in a turnover mixer or by hand at room temperature (about 25°C), centrifuge at 6,000 x g for 30 s at 4°C after turning over for 10 min, and then collect the supernatant into new EP tubes.
- 6. Antibody neutralisation: add a certain amount of neutralisation solution to the antibody eluate in step 5, generally 1/10 of the antibody elution volume (e.g., if the antibody eluate is 500 μL, the amount of neutralisation solution added is 50 μL), so that the pH value of the eluted antibody is maintained in a neutral environment, which is conducive to the maintenance of the biological activity of the antibody and the avoid antibody inactivation.
- 7. Post-treatment of Agarose: Wash the agarose twice with elution solution after use, centrifuge at 6,000 x g for 30 s at 4°C, and discard the supernatant; then wash three times with binding washing solution, centrifuge at 6,000 x g for 30 s at 4°C, and discard the supernatant; resuspend the agarose with 200 μL of preservation solution, and then store at 2~8°C.

- 1. Please read these instructions carefully before proceeding with antibody purification.
- 2. Do not freeze or centrifuge the agarose as this may cause irreversible aggregation of the agarose.

## Table 1

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
	lgG4	++++	++++
Human	IgA	++	-
	lgD	++	-
	IgE	++	-
	lgM	++	-
	lgG1	+	++++
	lgG2a	++++	++++
Mouse	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding



# Servicebio<sup>®</sup> SweAgrose Protein g Antibody Purified Agarose

## Cat. #: G2207

## **Product Information**

Product Name	Cat. No.	Spec.
Sucharasa Dratain C. Antihady Durifiad agarasa	G2207-1ML	1 mL
SweAgrose Protein & Antibody Purned agarose	G2207-5ML	5 mL

## Product Description/Introduction

Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42 kDa. Protein G is an immunoglobulin-binding protein expressed by Streptococcal bacteria (C or G). Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to agarose can be used for immunoprecipitation or antibody purification. Protein A agarose is suitable for binding human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, while Protein G agarose is suitable for binding human IgG1, IgG2, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c and other polyclonal antibodies. (See Table I for specific information) This product adopts the self-developed and produced Protein G labelled agarose, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc..

Characteristics	Description
Product content	50% (v/v) agarose in specific protective buffer
Beads structure	6% cross-linked agarose
Coupled protein	Protein G
M.W.of protein	~25 kDa(Protein G)
Binding capacity	>1mg mouse antibody per ml beads
Specificity	Antibodies from different species, including mice, humans, rats, goats, sheep and cattle
Beads size	30~150 μm
Elution method	Elution with acid, or 1x SDS-PAGE loading buffer (reduced) Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~ 50kDa) and light chain (~ 25kDa) of the antibody will be denatured and released from the agarose beads.
Application	IP, Co-IP, Protein purification

#### **Product Information**

## **Storage and Shipping Conditions**

Ship with wet ice; Store at 2-8°C, valid for 12 months.

#### **Product Components**

Component Number	Component	G2207-1ML	G2207-5ML
G2207	SweAgrose Protein G Antibody Purified agarose	1 mL	5 mL
Manual		1p	C

## **Experiment preparation**

Antibody purification related reagent formulations refer to the following, the user can be adjusted according to specific experimental conditions.

Component	Reagent combination		
Pinding Wash Puffer	PBST: 137 mM NaCl, 2.7 mM KCl, 10 mM Na <sub>2</sub> HPO <sub>4</sub> ,		
Binding wash Butter	2.0 mM KH <sub>2</sub> PO <sub>4</sub> , 0.1% Tween-20		
Elution Buffer	100 mM Gly, 0.1% Tween-20, pH 2.5		
Neutralization Buffer	1.0 M Tris-HCl, pH 9.0		
Preservation Buffer	PBST, 0.1% (v/v) Proclin 300		

## Manual procedure (purification of mouse ascites IgG as an example)

- 1. **Sample processing:** take 500 μL of ascites sample, add the binding wash buffer to make up 500 μL, if there are more protein precipitates in the sample, centrifuge the supernatant for experiments, which can improve the purity of the antibody.
- 2. Agarose pretreatment: Vortex the antibody purified agarose for 30 s to resuspend sufficiently, take 100 μL of 50% (v/v) SweAgarose Protein G Antibody Purified agarose in another new 1.5 mL EP tube, centrifuge at 6,000 x g for 30 s at 4 °C and discard the supernatant, wash it with 1 mL of binding washing buffer for twice, and then take the supernatant after magnetically aspirating.

Note: The amount of agarose can be adjusted according to the amount of antibody in the sample.

- 3. Antibody adsorption: add the sample processed in step 1 to the agarose in step 2, vortex and mix well, place the EP tube in a rotary mixer or manually turn the tube gently at room temperature (about 25°C) to make the agarose full contact with the sample, turn it over for 15 min, centrifuge at 6,000 x g for 30 s at 4°C, and then discard the supernatant.
- 4. **Agarose washing:** add 1 mL of binding wash buffer to the EP tube, resuspend with shaking and then centrifuge at 6,000 x g for 30 s at 4°C. Discard the supernatant and repeat the operation 3 times.
- 5. Antibody elution: Add 0.5~1.0 mL of eluent to the EP tubes with the agarose washed as described above, and resuspend the tubes rapidly by pipetting or vortexing, and then gently turn the tubes over in a turnover mixer or by hand at room temperature (about 25°C), centrifuge at 6,000 x g for 30 s at 4°C after turning over for 10 min, and then collect the supernatant into new EP tubes.
- 6. Antibody neutralisation: add a certain amount of neutralisation solution to the antibody eluate in step 5, generally 1/10 of the antibody elution volume (e.g., if the antibody eluate is 500 μL, the amount of neutralisation solution added is 50 μL), so that the pH value of the eluted antibody is maintained in a neutral environment, which is conducive to the maintenance of the biological activity of the antibody and the avoid antibody inactivation.
- 7. Post-treatment of Agarose: Wash the agarose twice with elution solution after use, centrifuge at 6,000 x g for 30 s at 4°C, and discard the supernatant; then wash three times with binding washing solution, centrifuge at 6,000 x g for 30 s at 4°C, and discard the supernatant; resuspend the agarose with 200 μL of preservation solution, and then store at 2~8°C.

- 1. Please read these instructions carefully before proceeding with antibody purification.
- 2. Do not freeze or centrifuge the agarose as this may cause irreversible aggregation of the agarose.

## Table 1

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
Llunaan	lgG4	++++	++++
Human	IgA	++	-
	IgD	++	-
	lgE	++	-
	lgM	++	-
	lgG1	+	++++
	lgG2a	++++	++++
Mouse	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding



## Servicebio<sup>®</sup> Sweagarose Protein a Antibody Purified Agarose

## Cat. #: G2206

## **Product Information**

Product Name	Cat. No.	Spec.
Swalarosa Drotain A Antihady Durified agarage	G2206-1ML	1 mL
SweAgrose Protein A Antibody Purned agarose	G2206-5ML	5 mL

## Product Description/Introduction

Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42 kDa. Protein G is an immunoglobulin-binding protein expressed by Streptococcal bacteria (C or G). Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to agarose can be used for immunoprecipitation or antibody purification. Protein A agarose is suitable for binding human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, etc., while Protein G agarose is suitable for binding human IgG1, IgG2, IgG4, mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c and other polyclonal antibodies. (See Table I for specific information)

This product adopts the self-developed and produced Protein A labelled agarose, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc..

Characteristics	Description	
Product content	50% (v/v) agarose in specific protective buffer	
Beads structure	6% cross-linked agarose	
Coupled protein	Protein A	
M.W.of protein	~25 kDa (Protein A)	
Binding capacity	>1mg mouse antibody per ml beads	
Specificity	Antibodies from different species, including mice, humans, rats, goats, sheep	
opeenery	and cattle	
Beads size	30~150 μm	
	Elution with acid, or 1x SDS-PAGE loading buffer (reduced)	
Elution method	Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~	
	50kDa) and light chain (~ 25kDa) of the antibody will be denatured and	
	released from the agarose beads.	
Application	IP, Co-IP, Protein purification	

#### **Product Information**

## **Storage and Shipping Conditions**

Ship with wet ice; Store at 2-8°C, valid for 12 months.

#### **Product Components**

Component Number	Component	G2206-1ML	G2206-5ML
G2206	SweAgrose Protein A Antibody Purified agarose	1 mL	5 mL
Manual		1	oc

## **Experiment preparation**

Antibody purification related reagent formulations refer to the following, the user can be adjusted according to specific experimental conditions.

Component	Reagent combination		
Pinding Wash Puffor	PBST: 137 mM NaCl, 2.7 mM KCl, 10 mM Na <sub>2</sub> HPO <sub>4</sub> ,		
binding wasn buner	2.0 mM KH <sub>2</sub> PO <sub>4</sub> , 0.1% Tween-20		
Elution Buffer	100 mM Gly, 0.1% Tween-20, pH 2.5		
Neutralization Buffer	1.0 M Tris-HCl, pH 9.0		
Preservation Buffer	PBST, 0.1% (v/v) Proclin 300		

## Manual procedure (purification of mouse ascites IgG as an example)

- Sample processing: take 500 μL of ascites sample, add the binding wash buffer to make up 500 μL, if there are more protein precipitates in the sample, centrifuge the supernatant for experiments, which can improve the purity of the antibody.
- 2. Agarose pretreatment: Vortex the antibody purified agarose for 30 s to resuspend sufficiently, take 100 μL of 50% (v/v) SweAgarose Protein A Antibody Purified agarose in another new 1.5 mL EP tube, centrifuge at 6,000 x g for 30 s at 4 °C and discard the supernatant, wash it with 1 mL of binding washing buffer for twice, and then take the supernatant after magnetically aspirating.

Note: The amount of agarose can be adjusted according to the amount of antibody in the sample.

- 3. Antibody adsorption: add the sample processed in step 1 to the agarose in step 2, vortex and mix well, place the EP tube in a rotary mixer or manually turn the tube gently at room temperature (about 25°C) to make the agarose full contact with the sample, turn it over for 15 min, centrifuge at 6,000 x g for 30 s at 4°C, and then discard the supernatant.
- 4. **Agarose washing:** add 1 mL of binding wash buffer to the EP tube, resuspend with shaking and then centrifuge at 6,000 x g for 30 s at 4°C. Discard the supernatant and repeat the operation 3 times.
- 5. Antibody elution: Add 0.5~1.0 mL of eluent to the EP tubes with the agarose washed as described above, and resuspend the tubes rapidly by pipetting or vortexing, and then gently turn the tubes over in a turnover mixer or by hand at room temperature (about 25°C), centrifuge at 6,000 x g for 30 s at 4°C after turning over for 10 min, and then collect the supernatant into new EP tubes.
- 6. Antibody neutralisation: add a certain amount of neutralisation solution to the antibody eluate in step 5, generally 1/10 of the antibody elution volume (e.g., if the antibody eluate is 500 μL, the amount of neutralisation solution added is 50 μL), so that the pH value of the eluted antibody is maintained in a neutral environment, which is conducive to the maintenance of the biological activity of the antibody and the avoid antibody inactivation.
- 7. Post-treatment of Agarose: Wash the agarose twice with elution solution after use, centrifuge at 6,000 x g for 30 s at 4°C, and discard the supernatant; then wash three times with binding washing solution, centrifuge at 6,000 x g for 30 s at 4°C, and discard the supernatant; resuspend the agarose with 200 μL of preservation solution, and then store at 2~8°C.

- 1. Please read these instructions carefully before proceeding with antibody purification.
- 2. Do not freeze or centrifuge the agarose as this may cause irreversible aggregation of the agarose.

## Table 1

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
Llunaan	lgG4	++++	++++
Human	IgA	++	-
	IgD	++	-
	lgE	++	-
	lgM	++	-
	lgG1	+	++++
	lgG2a	++++	++++
Mouse	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding



## Servicebio<sup>®</sup> Sweagarose Protein a Antibody Purified Agarose

## Cat. #: G2206

## **Product Information**

Product Name	Cat. No.	Spec.
Swalarosa Drotain A Antihady Durified agarage	G2206-1ML	1 mL
SweAgrose Protein A Antibody Purned agarose	G2206-5ML	5 mL

## Product Description/Introduction

Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42 kDa. Protein G is an immunoglobulin-binding protein expressed by Streptococcal bacteria (C or G). Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to agarose can be used for immunoprecipitation or antibody purification. Protein A agarose is suitable for binding human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, etc., while Protein G agarose is suitable for binding human IgG1, IgG2, IgG4, mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c and other polyclonal antibodies. (See Table I for specific information)

This product adopts the self-developed and produced Protein A labelled agarose, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc..

Characteristics	Description	
Product content	50% (v/v) agarose in specific protective buffer	
Beads structure	6% cross-linked agarose	
Coupled protein	Protein A	
M.W.of protein	~25 kDa (Protein A)	
Binding capacity	>1mg mouse antibody per ml beads	
Specificity	Antibodies from different species, including mice, humans, rats, goats, sheep	
opeenery	and cattle	
Beads size	30~150 μm	
	Elution with acid, or 1x SDS-PAGE loading buffer (reduced)	
Elution method	Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~	
	50kDa) and light chain (~ 25kDa) of the antibody will be denatured and	
	released from the agarose beads.	
Application	IP, Co-IP, Protein purification	

#### **Product Information**

## **Storage and Shipping Conditions**

Ship with wet ice; Store at 2-8°C, valid for 12 months.

#### **Product Components**

Component Number	Component	G2206-1ML	G2206-5ML
G2206	SweAgrose Protein A Antibody Purified agarose	1 mL	5 mL
Manual		1	oc

## **Experiment preparation**

Antibody purification related reagent formulations refer to the following, the user can be adjusted according to specific experimental conditions.

Component	Reagent combination		
Pinding Wash Puffor	PBST: 137 mM NaCl, 2.7 mM KCl, 10 mM Na <sub>2</sub> HPO <sub>4</sub> ,		
binding wasn buner	2.0 mM KH <sub>2</sub> PO <sub>4</sub> , 0.1% Tween-20		
Elution Buffer	100 mM Gly, 0.1% Tween-20, pH 2.5		
Neutralization Buffer	1.0 M Tris-HCl, pH 9.0		
Preservation Buffer	PBST, 0.1% (v/v) Proclin 300		

## Manual procedure (purification of mouse ascites IgG as an example)

- Sample processing: take 500 μL of ascites sample, add the binding wash buffer to make up 500 μL, if there are more protein precipitates in the sample, centrifuge the supernatant for experiments, which can improve the purity of the antibody.
- 2. Agarose pretreatment: Vortex the antibody purified agarose for 30 s to resuspend sufficiently, take 100 μL of 50% (v/v) SweAgarose Protein A Antibody Purified agarose in another new 1.5 mL EP tube, centrifuge at 6,000 x g for 30 s at 4 °C and discard the supernatant, wash it with 1 mL of binding washing buffer for twice, and then take the supernatant after magnetically aspirating.

Note: The amount of agarose can be adjusted according to the amount of antibody in the sample.

- 3. Antibody adsorption: add the sample processed in step 1 to the agarose in step 2, vortex and mix well, place the EP tube in a rotary mixer or manually turn the tube gently at room temperature (about 25°C) to make the agarose full contact with the sample, turn it over for 15 min, centrifuge at 6,000 x g for 30 s at 4°C, and then discard the supernatant.
- 4. **Agarose washing:** add 1 mL of binding wash buffer to the EP tube, resuspend with shaking and then centrifuge at 6,000 x g for 30 s at 4°C. Discard the supernatant and repeat the operation 3 times.
- 5. Antibody elution: Add 0.5~1.0 mL of eluent to the EP tubes with the agarose washed as described above, and resuspend the tubes rapidly by pipetting or vortexing, and then gently turn the tubes over in a turnover mixer or by hand at room temperature (about 25°C), centrifuge at 6,000 x g for 30 s at 4°C after turning over for 10 min, and then collect the supernatant into new EP tubes.
- 6. Antibody neutralisation: add a certain amount of neutralisation solution to the antibody eluate in step 5, generally 1/10 of the antibody elution volume (e.g., if the antibody eluate is 500 μL, the amount of neutralisation solution added is 50 μL), so that the pH value of the eluted antibody is maintained in a neutral environment, which is conducive to the maintenance of the biological activity of the antibody and the avoid antibody inactivation.
- 7. Post-treatment of Agarose: Wash the agarose twice with elution solution after use, centrifuge at 6,000 x g for 30 s at 4°C, and discard the supernatant; then wash three times with binding washing solution, centrifuge at 6,000 x g for 30 s at 4°C, and discard the supernatant; resuspend the agarose with 200 μL of preservation solution, and then store at 2~8°C.

- 1. Please read these instructions carefully before proceeding with antibody purification.
- 2. Do not freeze or centrifuge the agarose as this may cause irreversible aggregation of the agarose.

## Table 1

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
Llunaan	lgG4	++++	++++
Human	IgA	++	-
	IgD	++	-
	lgE	++	-
	lgM	++	-
	lgG1	+	++++
	lgG2a	++++	++++
Mouse	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding



## Servicebio<sup>®</sup> Immunoprecipitation Kit (Protein A/G Agarose)

## Cat. #:G2238

### **Product Information**

Product Name	Cat. No.	Spec.
Immunoprecipitation Kit (Protein A/G Agarose)	G2238-50T	50T

## Product Description/Introduction

IP or Co-IP is a common experimental technique for studying protein or protein-protein interactions (PPIs) by using specific antibodies and a medium that binds antibodies (e.g. Protein A/G Agarose or Protein A/G Magrose), or directly using a medium coupled with specific antibodies (e.g. agarose or magnetic beads), and then isolating the antigen-antibody complexes from the complicated protein samples by centrifugation or magnetic force, which can subsequently be used for Western blot or mass spectrometry. Protein G is an immunoglobulin binding protein expressed by Streptococcal bacteria type C or G. Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (lg). Recombinant Protein A and G with appropriate modifications bound to agarose can be used for immunoprecipitation or antibody purification. Protein A agarose are suitable for the immunoprecipitation of human lgG1, lgG2, lgG2a, lgG2b, while Protein G agarose beads are suitable for the immunoprecipitation of human lgG1, lgG2, lgG3, lgG4, mouse lgG1, lgG2a, lgG2b, lgG3, rat lgG1, lgG2a, lgG2b, lgG2c polyclonal antibodies. (See Table I for specific information)

This product adopts the self-developed and produced Protein A/G labelled agarose, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.; This kit provides two elution methods (denaturing elution and acid elution) to elute the target protein, especially the acid elution will not contain the light and heavy chains of the antibody, which can effectively solve the problem of the interference of the antibody's heavy and light chains in the immunoprecipitation and protein immunoblotting experiments.

Characteristics	Description	
Product Content	50%(v/v) agarose in specific protective buffer	
Beads structure	6% cross-linked agarose	
Coupled protein	Protein A/G	
M.W. of Protein	~25 kDa (Protein A/G)	
Binding protein capacity	>1mg mouse antibody per ml beads	
Specificity.	Antibodies from different species, including mice, humans, rats, goats, sheep	
Specificity	and cattle	
Bead Diameter	30~150 μm	
	Elution with acid, or 1x SDS-PAGE loading buffer (reduced)	
Elution Methods	Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~	
	50kDa) and light chain (~ 25kDa) of the antibody will be denatured and	
	released from the agarose beads.	
Applications	IP, Co-IP, Protein purification	

#### **Product Information**

## Storage and Shipping Conditions

Ship with wet ice; 5X SDS-PAGE loading buffer (reduced) should be stored at-20°C and other at 2-8°C for 12 months.

## Product Components

Component Number	Component	G2238-50T	Storage temperature
G2038	IP lysis buffer	50 mL	2-8°C
G0015	10×TBS	5 mL	2-8°C
G2238-1	SweAgarose Protein A/G	1 mL	2-8°C
G2238-2	Acid Elution Buffer	10 mL	2-8°C
G2238-3	Neulization Elution Buffer	1 mL	2-8°C
G2013	5x SDS-PAGE loading buffer (reduced)	1 mL	-20°C
Manual			1pc

## Additional Reagents Required

Product Name	Cat. Number	Spec.
50×Cocktail protease inhibitor	G2006-250UL	250 μL
PBS,1×(Phosphate Buffered Saline)	G4202-500ML	500 mL

### Assay Protocol/Procedures

## 1. Preparation of the kit

a) Refer to the table below, prepare the relevant reagents at a ratio of 100-500 μL of lysate per sample;

Step	Solution Required	Volum	Volum
Cell lysis and preparation	IP lysate containing Cocktail protease inhibitor	100 µL	500 μL
IP	SweAgarose Protein A/G	4 μL	20 µL
Wash three times	1×TBS	100 µL	500µL
Acid Elution &	Acid Elution Buffer	20 μL	100 μL
Neutralisation	Neutralization Buffer	20 µL	100 μL
Denaturing Elution	1x SDS-PAGE loading buffer (reduced)	20 μL	100 μL

- b) Preparation of IP lysate containing protease inhibitor: Refer to the above table, use 100-200 μL of IP lysate containing protease inhibitor per 0.5-1 million cells for lysis; mix the IP lysate with 50x Cocktail protease inhibitor (G2006-250UL is recommended) at a ratio of 50:1, For example, add 20 μL of 50x Cocktail protease inhibitor into 1 mL of IP lysate, then 1 mL of IP lysate containing protease inhibitor will be obtained; the prepared IP lysate containing the inhibitor should be placed in an ice bath or at 4°C
- c) Note: If the target protein of the immunoprecipitation involves phosphorylation or acetylation modification, phosphatase inhibitor or deacetylase inhibitor should be added (self-provided); IP lysate containing inhibitor should be prepared prior to use, and should not be frozen and retained for subsequent use.
- Preparation of 1×TBS: Dilute 10×TBS buffer with ultra-pure water to 1×TBS. For example, Add 1 mL of 10×TBS buffer to 9 mL of ultra-pure water, which is 1×TBS buffer after mix well.
- e) Preparation of 1x SDS-PAGE loading buffer (reduced): an appropriate amount of 5x SDS-PAGE loading buffer (reduced) was diluted 5 times with ultra-pure water to make 1x SDS-PAGE loading

buffer (reduced); for example, mix 0.2mL of 5x protein buffer (reduced) with 0.8 mL ultra-pure water, which is 1x SDS-PAGE loading buffer (reduced).

#### 2. Antigen sample preparation

Immunoprecipitation or immunoprecipitation experiments should be carried out immediately after samples lysed, if not, the samples can be stored in the refrigerator at  $-20^{\circ}$ C or  $-80^{\circ}$ C, but freezing and thawing may affect protein-protein interactions; all sample lysis steps should be operated in an ice bath or at 4°C in order to minimise the degradation of protein , and a certain amount of the sample should be taken as an Input or a Total after the sample has been prepared for subsequent detection by Western Blot.

For tissue samples:

- a) The tissues should be rinsed in pre-cold PBS (G4202 recommended) to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice.
- b) Add the IP lysate containing protease inhibitor at a ratio of 100-200 µL per 10-20 mg of tissue, or reduce the amount of IP lysate if higher concentrations of protein are required.
- c) Homogenise with a glass homogeniser or handheld homogeniser, or use our self-developed KZ-III-F low-temperature grinder for full grinding.
- d) The homogenate was transferred to a 1.5 mL centrifuge tube, shaken and mixed, and ice-bathed for 30 min, repeat blown with a pipette every 10 min to ensure complete lysis of the tissue cells.
- e) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For adherent cell samples:

- a) If necessary, the cells can be washed 2-3 times with PBS, absorb the residual liquid thoroughly at the last time.
- b) Scrape the cells with a cell scraper or trypsin digest the cells to make them fully suspended, collect them into a 1.5 mL centrifuge tube and centrifuge at 1,000 x g for 5 min at 4°C, discard the supernatant to collect the cell precipitate.
- c) Add 100-200 µL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells, and there should be no obvious cellular precipitation after fully lysed; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- d) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For suspend cell samples:

- a) Centrifuge at 1,000 x g for 5 min at 4°C to collect the precipitate; if necessary, wash once with PBS, then aspirate the residual liquid and gently vortex to disperse the cells as much as possible.
- b) Add 100-200 µL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- c) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For bacterial or yeast samples:

a) Take 1 mL of bacterial or yeast solution and centrifuge to remove the supernatant. If necessary, the cells can be washed once with PBS, absorb the residual liquid thoroughly at the last time.

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Gently vortex to disperse the cells as much as possible.

- b) Add 100-200 µL of IP lysate containing protease inhibitor and blow gently to fully disperse the bacteria or fungi.
- c) Ice bath for 10 min, for better lysis, bacteria and yeast can be digested with lysozyme and wall-breaking enzyme (self-provided) respectively, and then lysed with IP lysate containing protease inhibitors.
- d) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

#### 3. Agarose preparation

- a) Gently resuspend the SweAgarose Protein A/G beads to form a homogeneous bead dispersion as much as possible, add 20 µl of well-mixed bead dispersion for every 500 µl of sample, take an appropriate amount of agarose beads into a clean EP tube and add 1x TBS to a final volume of 0.5 mL (20 µl of bead dispersion for each sample is used in the following immunoprecipitation steps as an example).
- b) Gently resuspend the beads, centrifuge at  $6,000 \times g$  for 30s at 4 °C, carefully remove the supernatant and repeat the above steps twice.
- c) The beads were resuspended with 1x TBS according to the volume of the initial bead dispersion.

#### 4. Antibody binding to SweAgarose Protein A/G beads

- a) Antibody preparation: dilute the antibody with 1x TBS to prepare the antibody working solution according to the dilution ratio recommended in the antibody instructions, or prepare the antibody into an antibody working solution with a final concentration of 5-50  $\mu$ g/mL, which can be prepared on ice; optional: use normal IgG of the same antibody species to prepare normal IgG working solution with the same dilution ratio or final concentration of 5-50  $\mu$ g/mL, remove non-specific binding or serve as a negative control. The normal IgG of the same species means that if the antibody used in subsequent immunoprecipitation is mouse IgG, an appropriate amount of normal mouse IgG can be diluted with 1x TBS in this step to reduce the background or as a negative control.
- b) Antibody adsorption: Separate the SweAgarose Protein A/G beads prepared in step 3 by centrifuge at 6,000 x g for 30s at 4°C, aspirate the supernatant, add 500 µL of antibody working solution or normal IgG working solution, resuspend and then incubate for 15-60 min at room temperature on a turnover mixer.

Note: It is also possible to incubate the SweAgarose Protein A/G beads prepared in step 3 directly with an appropriate amount of antibody or normal IgG.

c) Beads separation and washing: Separate the incubated beads by centrifuge at 6,000 x g for 30s at 4°C, aspirate the supernatant, add 500 μL of 1×TBS, resuspend the SweAgarose Protein A/G beads by gently blowing with a pipette, centrifuge at 6,000 x g for 30s at 4°C, remove the supernatant, repeat the washing for three times, and resuspend the beads with 1×TBS in the same amount as the initial volume.

Note: If the beads are agglomerated or flaky during incubation and washing, it is a normal phenomenon and will not affect the experimental results.

#### 5. Immunoprecipitation (IP):

a) Removal of non-specific binding (optional): the SweAgarose Protein A/G beads prepared in step 4, which bind normal IgG, are incubated with the samples at 4°C for 60 min and separated by centrifuge at 6,000 x g for 30s at 4 °C, and the supernatants are used for the subsequent

experiments; the purpose of this step is to remove the proteins that bind non-specifically to normal IgG.

b) Incubation of samples with SweAgarose Protein A/G beads conjugated with antibody or normal IgG: add SweAgarose Protein A/G beads conjugated with antibody or normal IgG at the ratio of 20 µl of bead suspension for every 500 µl of protein sample, place on a side-oscillating shaker or a rotary mixer, and incubate for 2 hours at room temperature or overnight at 4°C.

Note 1: If the beads are agglomerated or flaky during incubation and washing, it is a normal phenomenon and will not affect the experimental results.

Note 2: Alternatively, the appropriate amount of antibody or normal IgG can be incubated with the sample for 1-2 hours at room temperature or overnight at 4°C, and then 10-20  $\mu$ L of agarose bead suspension can be added and incubated for 60 min at room temperature.

- c) Centrifuge: after incubation, centrifuge at 6,000 x g for 30s at 4°C to remove the supernatant.
  Note: Retain a portion of the supernatant to test the effect of immunoprecipitation.
- d) Wash: Add 500 µl of 1×TBS, gently blow the resuspended beads with a pipette, centrifuge at 6,000 x g for 30s at 4°C to remove the supernatant and repeat the wash three times.
  Note: You can also assay the OD280 of the washing buffer to determine whether the washing is complete, if the OD280 is more than 0.05, the number of washing times should be increased appropriately.

#### 6. Elution

Depending on the characteristics of the target protein and the requirements of subsequent experiments, one of the following methods can be selected for elution.

a) **Denaturing elution method:** Samples eluted by this method are suitable for SDS-PAGE. Add 25  $\mu$ L of 1× Protein Sampling Buffer (Reduced) to the tube after centrifuge, heat at 95°C for 5 min, and then centeifuge to collect the supernatant for Western blot detection.

b) Acid Elution: The sample eluted by this method retains its original biological activity and can be used for post-functional analysis. Add 20  $\mu$ L of Acid Elution Buffer to the tube after centrifuge, mix well and incubate at room temperature for 10 min, then centrifuge to collect the supernatant into a new EP tube, and immediately add 2  $\mu$ L of Neulization Elution Buffer to adjust the pH of the eluted product to neutral, which can be used for post-functional analysis.

Note: The user can adjust the amount of elution buffer added according to the desired volume.

- 1. Do not store SweAgarose Protein A/G in the kit at -20°C or freeze and thaw repeatedly, otherwise it will affect the performance of magnetic beads and cause unnecessary experimental errors.
- 2. Please read these instructions carefully before performing the immunoprecipitation procedure.
- 3. If the immunoprecipitated target protein involves phosphorylation or acetylation modification, appropriate phosphatase inhibitors and deacetylase inhibitors should be provided.
- 4. Maintain beads at pH 6-8, avoid high speed centrifugation, drying or freezing, which may cause agglomeration of the beads.
- 5. The beads should be mixed thoroughly before use, the mixing operation should be gentle and should not be subjected to violent swirling and shaking to avoid denaturation of the antibody.
- 6. Positive and negative controls (SweAgarose Mouse IgG) are recommended in immunoprecipitation.
- 7. Protein samples should be purified as soon as possible after collection and always placed at 4°C or in an ice bath to slow down protein degradation or denaturation.



- 8. Agarose beads may gather when use with acid elution, which is a normal phenomenon and does not affect the normal use of magnetic beads.
- 9. For research use only. Not for use in diagnostic or therapeutic procedures.
- 10. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
- 11. The binding capacity of Protein A and Protein G to antibodies of different generic sources and subtypes is shown in Table I.

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
Liveren	lgG4	++++	++++
Human	IgA	++	-
	IgD	++	-
	IgE	++	-
	lgM	++	-
Mouse	lgG1	+	++++
	lgG2a	++++	++++
	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
	lgG1	-	+
	lgG2a	-	++++
Rat	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Tabl	e I
------	-----

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding



# Servicebio<sup>®</sup> Immunoprecipitation Kit (Protein A/G Magnetic Beads) Cat. #: G2237

### **Product Information**

Product Name	Cat. No.	Spec.
Immunoprecipitation Kit (Protein A/G Magnetic Beads)	G2237-50T	50T

## Product Description/Introduction

IP or Co-IP is a common experimental technique for studying protein or protein-protein interactions (PPIs) by using specific antibodies and a medium that binds antibodies (e.g. Protein A/G Agarose or Protein A/G magnetic beads), or directly using a medium coupled with specific antibodies (e.g. agarose gels or magnetic beads), and then isolating the antigen-antibody complexes from the complicated protein samples by centrifugation or magnetic force, which can subsequently be used for Western blot or mass spectrometry. Protein G is an immunoglobulin binding protein expressed by *Streptococcal bacteria* type C or G. Protein A is a cell wall surface protein found in *Staphylococcus aureus* with a molecular weight of 42kDa. Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to magnetic beads can be used for immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, while Protein G magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, mouse IgG1, IgG2a, IgG2b, IgG2b, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c polyclonal antibodies. (See Table I for specific information)

This product adopts the self-developed and produced Protein A/G protein-labelled magnetic beads, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.; This kit provides two elution methods (denaturing elution and acid elution) to elute the target protein, especially the acid elution will not contain the light and heavy chains of the antibody, which can effectively solve the problem of the interference of the antibody's heavy and light chains in the immunoprecipitation and protein immunoblotting experiments.

Characteristics	Description	
Product Content	10%(v/v) magnetic beads in specific protective buffer	
Magnetization	Superparamagnetism	
Coupled protein	Protein A/G	
M.W. of Protein	~25 kDa (Protein A/G)	
Binding protein capacity	>1mg mouse antibody per ml beads	
Specificity	Antibodies from different species, including mice, humans, rats, goats, sheep and cattle	
Bead Diameter	30~150 μm	
Elution Methods	Elution with acid, or 1x SDS-PAGE loading buffer (reduced) Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~ 50kDa) and light chain (~ 25kDa) of the antibody will be denatured and released from the magnetic beads.	

#### **Product Information**



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IP, Co-IP, Protein purification

## Storage and Shipping Conditions

Ship with wet ice; 5X SDS-PAGE loading buffer (reduced) should be stored at-20  $^{\circ}$ C and other at 2-8 $^{\circ}$ C for 12 months.

## **Product Components**

Component Number	Component	G2237-50T	Storage temperature
G2038	IP lysis buffer	50 mL	2-8°C
G0015	10×TBS	5 mL	2-8°C
G2237-1	SweMagrose Protein A	1 mL	2-8°C
G2237-2	Acid Elution Buffer	10 mL	2-8°C
G2237-3	Neulization Elution Buffer	1 mL	2-8°C
G2013	5x SDS-PAGE loading buffer (reduced)	1 mL	-20°C
Manual			1 pc

## **Additional Reagents Required**

Product Name	Cat. Number	Spec.
50×Cocktail protease inhibitor	G2006-250UL	250 μL
PBS,1×(Phosphate Buffered Saline)	G4202-500ML	500 mL

## Assay Protocol/Procedures

## 1. Preparation of the kit

a) Refer to the table below, prepare the relevant reagents at a ratio of 100-500  $\,\mu L$  of lysate per sample;

Step	Solution Required	Volum	Volum
Cell lysis and	IP lysate containing Cocktail	100l	500 ul
preparation	protease inhibitor	100 με	500 με
IP	SweMagrose Protein A/G	4 μL	20 μL
Wash three times	1×TBS	100 μL	500µL
Acid Elution &	Acid Elution Buffer	20 µL	100 μL
Neutralisation	Neutralization Buffer	20 µL	100 μL
Denaturing Elution	1x SDS-PAGE loading buffer (reduced)	20 μL	100 µL

- b) Preparation of IP lysate containing protease inhibitor: Refer to the above table, use 100-200 μL of IP lysate containing protease inhibitor per 0.5-1 million cells for lysis; mix the IP lysate with 50x Cocktail protease inhibitor (G2006-250UL is recommended) at a ratio of 50:1, For example, add 20 μL of 50x Cocktail protease inhibitor into 1 mL of IP lysate, then 1 mL of IP lysate containing protease inhibitor will be obtained; the prepared IP lysate containing the inhibitor should be placed in an ice bath or at 4°C.
- c) Note: If the target protein of the immunoprecipitation involves phosphorylation or acetylation modification, phosphatase inhibitor or deacetylase inhibitor should be added (self-provided); IP lysate containing inhibitor should be prepared prior to use, and should not be frozen and retained for subsequent use.
- d) Preparation of 1×TBS: Dilute 10×TBS buffer with ultra-pure water to 1×TBS. For example, Add 1
  mL of 10×TBS buffer to 9 mL of ultra-pure water, which is 1×TBS buffer after mix well.
- e) Preparation of 1x SDS-PAGE loading buffer (reduced): an appropriate amount of 5x SDS-PAGE

loading buffer (reduced) was diluted 5 times with ultra-pure water to make 1x SDS-PAGE loading buffer (reduced); for example, mix 0.2mL of 5x protein buffer (reduced) with 0.8 mL ultra-pure water, which is 1x SDS-PAGE loading buffer (reduced).

#### 2. Antigen sample preparation

Immunoprecipitation or immunoprecipitation experiments should be carried out immediately after samples lysed, if not, the samples can be stored in the refrigerator at  $-20^{\circ}$ C or  $-80^{\circ}$ C, but freezing and thawing may affect protein-protein interactions; all sample lysis steps should be operated in an ice bath or at 4°C in order to minimise the degradation of protein , and a certain amount of the sample should be taken as an Input or a Total after the sample has been prepared for subsequent detection by Western Blot.

For tissue samples:

- a) The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice.
- b) Add the IP lysate containing protease inhibitor at a ratio of 100-200 µL per 10-20 mg of tissue, or reduce the amount of IP lysate if higher concentrations of protein are required.
- Homogenise with a glass homogeniser or handheld homogeniser, or use our self-developed KZ-III-F low-temperature grinder for full grinding.
- d) The homogenate was transferred to a 1.5 mL centrifuge tube, shaken and mixed, and ice-bathed for 30 min, repeat blown with a pipette every 10 min to ensure complete lysis of the tissue cells.
- e) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For adherent cell samples:

- a) If necessary, the cells can be washed 2-3 times with PBS, absorb the residual liquid thoroughly at the last time.
- b) Scrape the cells with a cell scraper or trypsin digest the cells to make them fully suspended, collect them into a 1.5 mL centrifuge tube and centrifuge at 1,000 x g for 5 min at 4°C, discard the supernatant to collect the cell precipitate.
- c) Add 100-200 µL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells, and there should be no obvious cellular precipitation after fully lysed; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- d) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For suspend cell samples:

- a) Centrifuge at 1000 g for 5 min at 4°C to collect the precipitate; if necessary, wash once with PBS, then aspirate the residual liquid and gently vortex to disperse the cells as much as possible.
- b) Add 100-200 µL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- c) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For bacterial or yeast samples:

a) Take 1 mL of bacterial or yeast solution and centrifuge to remove the supernatant. If necessary,

the cells can be washed once with PBS, absorb the residual liquid thoroughly at the last time. Gently vortex to disperse the cells as much as possible.

- b) Add 100-200 µL of IP lysate containing protease inhibitor and blow gently to fully disperse the bacteria or fungi.
- c) Ice bath for 10 min, for better lysis, bacteria and yeast can be digested with lysozyme and wall-breaking enzyme (self-provided) respectively, and then lysed with IP lysate containing protease inhibitors.
- d) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

#### 3. Magnetic bead preparation

- a) Gently resuspend the SweMagrose Protein A/G magnetic beads to form a homogeneous bead dispersion as much as possible, add 20 µl of well-mixed bead dispersion for every 500 µl of sample, take an appropriate amount of magnetic beads into a clean EP tube and add 1x TBS to a final volume of 0.5 mL (20 µl of bead dispersion for each sample is used in the following immunoprecipitation steps as an example).
- b) Gently resuspend the magnetic beads, place on a magnetic separation stand for 30 s, carefully remove the supernatant and repeat the above steps twice.
- c) The magnetic beads were resuspended with 1x TBS according to the volume of the initial bead dispersion.

#### 4. Antibody binding to SweMagrose Protein A/G magnetic beads

- a) Antibody preparation: dilute the antibody with 1x TBS to prepare the antibody working solution according to the dilution ratio recommended in the antibody instructions, or prepare the antibody into an antibody working solution with a final concentration of 5-50  $\mu$ g/mL, which can be prepared on ice; **optional:** use normal IgG of the same antibody species to prepare normal IgG working solution with the same dilution ratio or final concentration of 5-50  $\mu$ g/mL, remove non-specific binding or serve as a negative control. The normal IgG of the same species means that if the antibody used in subsequent immunoprecipitation is mouse IgG, an appropriate amount of normal mouse IgG can be diluted with 1x TBS in this step to reduce the background or as a negative control.
- b) Antibody adsorption: Separate the SweMagrose Protein A/G magnetic beads prepared in step 3 by magnetic, aspirate the supernatant, add 500 µL of antibody working solution or normal IgG working solution, resuspend and then incubate for 15-60 min at room temperature on a turnover mixer.

# Note: It is also possible to incubate the SweMagrose Protein A/G magnetic beads prepared in step 3 directly with an appropriate amount of antibody or normal IgG.

c) Magnetic separation and washing: Separate the incubated beads by magnetic, aspirate the supernatant, add 500  $\mu$ L of 1×TBS, resuspend the SweMagrose Protein A/G beads by gently blowing with a pipette, place on a magnetic separation rack for 10 s, remove the supernatant, repeat the washing for three times, and resuspend the beads with 1×TBS in the same amount as the initial volume.

Note: If the beads are agglomerated or flaky during incubation and washing, it is a normal phenomenon and will not affect the experimental results.

#### 5. Immunoprecipitation (IP) :

a) Removal of non-specific binding (optional): the SweMagrose Protein A/G beads prepared in step

4, which bind normal IgG, are incubated with the samples at 4°C for 60 min and separated by magnetic suction, and the supernatants are used for the subsequent experiments; the purpose of this step is to remove the proteins that bind non-specifically to normal IgG.

b) Incubation of samples with SweMagrose Protein A/G beads conjugated with antibody or normal IgG: add SweMagrose Protein A/G beads conjugated with antibody or normal IgG at the ratio of 20 µl of bead suspension for every 500 µl of protein sample, place on a side-oscillating shaker or a rotary mixer, and incubate for 2 hours at room temperature or overnight at 4°C.

Note 1: If the beads are agglomerated or flaky during incubation and washing, it is a normal phenomenon and will not affect the experimental results.

Note 2: Alternatively, the appropriate amount of antibody or normal IgG can be incubated with the sample for 1-2 hours at room temperature or overnight at 4°C, and then 10-20  $\mu$ L of magnetic bead suspension can be added and incubated for 60 min at room temperature.

c) Magnetic separation: after incubation, place on a magnetic separation stand for 10 seconds to remove the supernatant.

Note: Retain a portion of the supernatant to test the effect of immunoprecipitation.

d) Wash: Add 500 µl of 1 × TBS, gently blow the resuspended beads with a pipette, place on a magnetic separator for 10 seconds to remove the supernatant and repeat the wash three times.
 Note: You can also test the OD280 of the washing solution to determine whether the washing is complete, if the OD280 is more than 0.05, the number of washing times should be increased appropriately.

#### 6. Elution

Depending on the characteristics of the target protein and the requirements of subsequent experiments, one of the following methods can be selected for elution.

a) **Denaturing elution method:** Samples eluted by this method are suitable for SDS-PAGE. Remove the EP tube from the magnetic separator, add 25  $\mu$ L of 1× Protein Sampling Buffer (Reduced) to the tube, heat at 95°C for 5 min, and then perform a magnetic separation to collect the supernatant for Western blot detection.

b) Acid Elution: The sample eluted by this method retains its original biological activity and can be used for post-functional analysis. Remove the EP tube from the magnetic separation rack, add 20  $\mu$ L of Acid Elution Buffer to the tube, mix well and incubate at room temperature for 10 min, then perform a magnetic suction separation, collect the supernatant into a new EP tube, and immediately add 2  $\mu$ L of Neulization Elution Buffer to adjust the pH of the eluted product to neutral, which can be used for post-functional analysis.

# Note: The user can adjust the amount of elution buffer added according to the desired volume.

- 1. Do not store SweMagrose Protein A/G in the kit at -20°C or freeze and thaw repeatedly, otherwise it will affect the performance of magnetic beads and cause unnecessary experimental errors.
- 2. Please read these instructions carefully before performing the immunoprecipitation procedure.
- 3. Magnetic separation frame for magnetic separation need self-provided
- 4. If the immunoprecipitated target protein involves phosphorylation or acetylation modification, appropriate phosphatase inhibitors and deacetylase inhibitors should be provided.
- Maintain beads at pH 6-8, avoid high speed centrifugation, drying or freezing; do not expose beads to magnetic fields for long periods of time which may cause agglomeration of the beads.



- 6. The beads should be mixed thoroughly before use, the mixing operation should be gentle and should not be subjected to violent swirling and shaking to avoid denaturation of the antibody.
- 7. Positive and negative controls (SweMagrose Mouse IgG) are recommended in immunoprecipitation.
- 8. Protein samples should be purified as soon as possible after collection and always placed at 4°C or in an ice bath to slow down protein degradation or denaturation
- 9. Magnetic beads may gather when use with acid elution, which is a normal phenomenon and does not affect the normal use of magnetic beads.
- 10. For research use only. Not for use in diagnostic or therapeutic procedures.
- 11. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
- 12. The binding capacity of Protein A and Protein G to antibodies of different generic sources and subtypes is shown in Table I.

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
Liveren	lgG4	++++	++++
Human	IgA	++	-
	IgD	++	-
	IgE	++	-
	lgM	++	-
	lgG1	+	++++
	lgG2a	++++	++++
Mouse	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

#### Table 1

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

- +: Weak Binding
- +/-: Weak or No Binding

-:No Binding



## Servicebio<sup>®</sup> Immunoprecipitation Kit (Protein G Agarose)

## Cat. #:G2216

#### **Product Information**

Product Name	Cat. No.	Spec.
Immunoprecipitation Kit (Protein G Agarose)	G2216-50T	50 T

## Product Description/Introduction

IP or Co-IP is a common experimental technique for studying protein or protein-protein interactions (PPIs) by using specific antibodies and a medium that binds antibodies (e.g. Protein A/G Agarose or Protein A/G Magrose), or directly using a medium coupled with specific antibodies (e.g. agarose or magnetic beads), and then isolating the antigen-antibody complexes from the complicated protein samples by centrifugation or magnetic force, so as to achieve the purpose of isolation and purification of the target proteins, which can subsequently be used for Western blot or mass spectrometry. Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42 kDa. Protein G is an immunoglobulin binding protein expressed by Streptococcal bacteria type C or G. Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to agarose can be used for immunoprecipitation or antibody purification. Protein A agarose are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, while Protein G agarose beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG2a

This product adopts the self-developed and produced Protein G labelled agarose, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.; This kit provides two elution methods (denaturing elution and acid elution) to elute the target protein, especially the acid elution will not contain the light and heavy chains of the antibody, which can effectively solve the problem of the interference of the antibody's heavy and light chains in the immunoprecipitation and protein immunoblotting experiments.

Characteristics	Description	
Product Content	50%(v/v) agarose in specific protective buffer	
Beads structure	6% cross-linked agarose	
Coupled protein	Protein G	
M.W. of Protein	~25 kDa (Protein G)	
Binding protein capacity	1mg mouse antibody per ml beads	
Specificity	Antibodies from different species, including mice, humans, rats, goats, sheep	
Specificity	and cattle	
Bead Diameter	30~150 μm	
	Elution with acid, or 1x SDS-PAGE loading buffer (reduced)	
Elution Methods	Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~	
	50kDa) and light chain (~ 25kDa) of the antibody will be denatured and	
	released from the agarose beads.	
Applications	IP, Co-IP, Protein purification	

#### **Product Information**

## Storage and Shipping Conditions

Ship with wet ice; 5×SDS-PAGE loading buffer (reduced) should be stored at -20°C and other at 2-8°C for 12 months.

## Product Components

Component Number	Component	G2216-50T	Storage temperature
G2038	IP lysis buffer	50 mL	2-8°C
G0015	10×TBS	5 mL	2-8°C
G2216-1	SweAgarose Protein G	1 mL	2-8°C
G2216-2	Acid Elution Buffer	10 mL	2-8°C
G2216-3	Neulization Elution Buffer	1 mL	2-8°C
G2013	5x SDS-PAGE loading buffer (reduced)	1 mL	-20°C
Manual			1pc

## Additional Reagents Required

Product Name	Cat. Number	Spec.
50×Cocktail protease inhibitor	G2006-250UL	250 μL
PBS,1×(Phosphate Buffered Saline)	G4202-500ML	500 mL

## Assay Protocol/Procedures

## 1. Preparation of the kit

a) Refer to the table below, prepare the relevant reagents at a ratio of 100-500 μL of lysate per sample;

Step	Solution Required	Volum	Volum	
Cell lysis and	IP lysate containing Cocktail	100 uL	500 uL	
preparation	protease inhibitor		P	
IP	SweAgarose Protein G	4 μL	20 μL	
Wash three times	1×TBS	100 µL	500µL	
Acid Elution &	Acid Elution Buffer	20 µL	100 μL	
Neutralisation	Neutralization Buffer	20 µL	100 µL	
Denaturing Elution	1x SDS-PAGE loading buffer (reduced)	20 µL	100 μL	

- b) Preparation of IP lysate containing protease inhibitor: Refer to the above table, use 100-200 μL of IP lysate containing protease inhibitor per 0.5-1 million cells for lysis; mix the IP lysate with 50x Cocktail protease inhibitor (G2006-250UL is recommended) at a ratio of 50:1, for example, add 20 μL of 50x Cocktail protease inhibitor into 1 mL of IP lysate, then 1 mL of IP lysate containing protease inhibitor will be obtained; the prepared IP lysate containing the inhibitor should be placed in an ice bath or at 4°C
- c) Note: If the target protein of the immunoprecipitation involves phosphorylation or acetylation modification, phosphatase inhibitor or deacetylase inhibitor should be added (self-provided); IP lysate containing inhibitor should be prepared prior to use, and should not be frozen and retained for subsequent use.
- Preparation of 1×TBS: Dilute 10×TBS buffer with ultra-pure water to 1×TBS. For example, Add 1 mL of 10×TBS buffer to 9 mL of ultra-pure water, which is 1×TBS buffer after mix well.
- e) Preparation of 1x SDS-PAGE loading buffer (reduced): an appropriate amount of 5x SDS-PAGE loading buffer (reduced) was diluted 5 times with ultra-pure water to make 1x SDS-PAGE loading
buffer (reduced); for example, mix 0.2mL of 5x protein buffer (reduced) with 0.8 mL ultra-pure water, which is 1x SDS-PAGE loading buffer (reduced).

#### 2. Antigen sample preparation

Immunoprecipitation or immunoprecipitation experiments should be carried out immediately after samples lysed, if not, the samples can be stored in the refrigerator at  $-20^{\circ}$ C or  $-80^{\circ}$ C, but freezing and thawing may affect protein-protein interactions; all sample lysis steps should be operated in an ice bath or at 4°C in order to minimise the degradation of protein , and a certain amount of the sample should be taken as an Input or a Total after the sample has been prepared for subsequent detection by Western Blot.

For tissue samples:

- a) The tissues should be rinsed in pre-cold PBS (G4202 recommended) to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice.
- b) Add the IP lysate containing protease inhibitor at a ratio of 100-200 µL per 10-20 mg of tissue, or reduce the amount of IP lysate if higher concentrations of protein are required.
- c) Homogenise with a glass homogeniser or handheld homogeniser, or use our self-developed KZ-III-F low-temperature grinder for full grinding.
- d) The homogenate was transferred to a 1.5 mL centrifuge tube, shaken and mixed, and ice-bathed for 30 min, repeat blown with a pipette every 10 min to ensure complete lysis of the tissue cells.
- e) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For adherent cell samples:

- a) If necessary, the cells can be washed 2-3 times with PBS, absorb the residual liquid thoroughly at the last time.
- b) Scrape the cells with a cell scraper or trypsin digest the cells to make them fully suspended, collect them into a 1.5 mL centrifuge tube and centrifuge at 1,000 x g for 5 min at 4°C, discard the supernatant to collect the cell precipitate.
- c) Add 100-200 µL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells, and there should be no obvious cellular precipitation after fully lysed; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- d) After sufficient lysis, centrifuge at 12,000 x g for 5 minutes at 4 ℃, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For suspend cell samples:

- a) Centrifuge at 1,000 x g for 5 min at 4°C to collect the precipitate; if necessary, wash once with PBS, then aspirate the residual liquid and gently vortex or flick the bottom of the tube to disperse the cells as much as possible.
- b) Add 100-200 µL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- c) Ice bath for 5 min, centrifugation at 12000 g 4°C for 5 min, take the supernatant, that is, the total protein solution, which can be used for subsequent immunoprecipitation or immunoprecipitation experiments and so on.

For bacterial or yeast samples:

- a) Take 1 mL of bacterial or yeast solution and centrifuge to remove the supernatant. If necessary, the cells can be washed once with PBS, absorb the residual liquid thoroughly at the last time. Gently vortex or flick the bottom of the tube to disperse the cells as much as possible.
- b) Add 100-200 µL of IP lysate containing protease inhibitor and blow gently to fully disperse the bacteria or fungi.
- c) Ice bath for 10 min, for better lysis, bacteria and yeast can be digested with lysozyme and wall-breaking enzyme (self-provided) respectively, and then lysed with IP lysate containing protease inhibitors.
- d) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

#### 3. Agarose preparation

- a) Gently resuspend the SweAgarose Protein G beads to form a homogeneous bead dispersion as much as possible, add 20 µl of well-mixed bead dispersion for every 500 µl of sample, take an appropriate amount of agarose beads into a clean EP tube and add 1x TBS to a final volume of 0.5 mL (20 µl of bead dispersion for each sample is used in the following immunoprecipitation steps as an example).
- b) Gently resuspend the beads, centrifuge at  $6,000 \times g$  for 30s at 4 °C, carefully remove the supernatant and repeat the above steps twice.
- c) The beads were resuspended with 1x TBS according to the volume of the initial bead dispersion.

#### 4. Antibody binding to SweAgarose Protein G beads

- a) Antibody preparation: dilute the antibody with 1x TBS to prepare the antibody working solution according to the dilution ratio recommended in the antibody instructions, or prepare the antibody into an antibody working solution with a final concentration of 5-50  $\mu$ g/mL, which can be prepared on ice; optional: use normal IgG of the same antibody species to prepare normal IgG working solution with the same dilution ratio or final concentration of 5-50  $\mu$ g/mL, remove non-specific binding or serve as a negative control. The normal IgG of the same species means that if the antibody used in subsequent immunoprecipitation is mouse IgG, an appropriate amount of normal mouse IgG can be diluted with 1x TBS in this step to reduce the background or as a negative control.
- b) Antibody adsorption: Separate the SweAgarose Protein G beads prepared in step 3 by centrifuge at 6,000 x g for 30s at 4°C, aspirate the supernatant, add 500 µL of antibody working solution or normal IgG working solution, resuspend and then incubate for 15-60 min at room temperature on a turnover mixer.

# Note: It is also possible to incubate the SweAgarose Protein G beads prepared in step 3 directly with an appropriate amount of antibody or normal IgG.

c) Beads separation and washing: Separate the incubated beads by centrifuge at 6,000 x g for 30s at 4°C, aspirate the supernatant, add 500  $\mu$ L of 1×TBS, resuspend the SweAgarose Protein G beads by gently blowing with a pipette, centrifuge at 6,000 x g for 30s at 4°C, remove the supernatant, repeat the washing for three times, and resuspend the beads with 1×TBS in the same amount as the initial volume.

Note: If the beads are agglomerated or flaky during incubation and washing, it is a normal phenomenon and will not affect the experimental results.

#### 5. Immunoprecipitation (IP):

a) Removal of non-specific binding (optional): the SweAgarose Protein G beads prepared in step 4,

which bind normal IgG, are incubated with the samples at  $4^{\circ}$ C for 60 min and separated by centrifuge at 6,000 x g for 30s at  $4^{\circ}$ C, and the supernatants are used for the subsequent experiments; the purpose of this step is to remove the proteins that bind non-specifically to normal IgG.

b) Incubation of samples with SweAgarose Protein G beads conjugated with antibody or normal IgG: add SweAgarose Protein G beads conjugated with antibody or normal IgG at the ratio of 20 µl of bead suspension for every 500 µl of protein sample, place on a side-oscillating shaker or a rotary mixer, and incubate for 2 hours at room temperature or overnight at 4°C.

Note 1: If the beads are agglomerated or flaky during incubation and washing, it is a normal phenomenon and will not affect the experimental results.

Note 2: Alternatively, the appropriate amount of antibody or normal IgG can be incubated with the sample for 1-2 hours at room temperature or overnight at 4°C, and then 10-20  $\mu$ L of agarose bead suspension can be added and incubated for 60 min at room temperature.

- c) Centrifuge: after incubation, centrifuge at 6,000 x g for 30s at 4°C to remove the supernatant.
  Note: Retain a portion of the supernatant to test the effect of immunoprecipitation.
- d) Wash: Add 500 µl of 1×TBS, gently blow the resuspended beads with a pipette, centrifuge at 6,000 x g for 30s at 4°C to remove the supernatant and repeat the wash three times.
  Note: You can also assay the OD280 of the washing buffer to determine whether the washing is complete, if the OD280 is more than 0.05, the number of washing times should be increased appropriately.

#### 6. Elution

Depending on the characteristics of the target protein and the requirements of subsequent experiments, one of the following methods can be selected for elution.

a) **Denaturing elution method:** Samples eluted by this method are suitable for SDS-PAGE. Add 25  $\mu$ L of 1× Protein Sampling Buffer (Reduced) to the tube after centrifuge, heat at 95°C for 5 min, and then centeifuge to collect the supernatant for Western blot detection.

b) Acid Elution: The sample eluted by this method retains its original biological activity and can be used for post-functional analysis. Add 20  $\mu$ L of Acid Elution Buffer to the tube after centrifuge, mix well and incubate at room temperature for 10 min, then centrifuge to collect the supernatant into a new EP tube, and immediately add 2  $\mu$ L of Neulization Elution Buffer to adjust the pH of the eluted product to neutral, which can be used for post-functional analysis.

Note: The user can adjust the amount of elution buffer added according to the desired volume.

#### Note

- 1. Do not store SweAgarose Protein G in the kit at -20°C or freeze and thaw repeatedly, otherwise it will affect the performance of magnetic beads and cause unnecessary experimental errors.
- 2. Please read these instructions carefully before performing the immunoprecipitation procedure.
- 3. If the immunoprecipitated target protein involves phosphorylation or acetylation modification, appropriate phosphatase inhibitors and deacetylase inhibitors should be provided.
- 4. Maintain beads at pH 6-8, avoid high speed centrifugation, drying or freezing, which may cause agglomeration of the beads.
- 5. The beads should be mixed thoroughly before use, the mixing operation should be gentle and should not be subjected to violent swirling and shaking to avoid denaturation of the antibody.
- 6. Positive and negative controls (SweAgarose Mouse IgG) are recommended in immunoprecipitation.

- 7. Protein samples should be purified as soon as possible after collection and always placed at 4°C or in an ice bath to slow down protein degradation or denaturation.
- 8. Agarose beads may gather when use with acid elution, which is a normal phenomenon and does not affect the normal use of magnetic beads.
- 9. This product is restricted to scientific research use by professionals, and is not to be used for clinical diagnosis or treatment, food or medicine, or stored in an ordinary residence.
- 10. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
- 11. The binding capacity of Protein A and Protein G to antibodies of different generic sources and subtypes is shown in Table I.

#### Table I

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
	lgG4	++++	++++
Human	IgA	++	-
	lgD	++	-
	IgE	++	-
	lgM	++	-
Mouse	lgG1	+	++++
	lgG2a	++++	++++
	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
	lgG1	-	+
Rat	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

- +: Weak Binding
- +/-: Weak or No Binding
- -:No Binding



# Servicebio<sup>®</sup> Immunoprecipitation Kit (Protein A Agarose)

# Cat. #:G2215

#### **Product Information**

Product Name	Cat. No.	Spec.
Immunoprecipitation Kit (Protein A Agarose)	G2215-50T	50 T

## Product Description/Introduction

IP or Co-IP is a common experimental technique for studying protein or protein-protein interactions (PPIs) by using specific antibodies and a medium that binds antibodies (e.g. Protein A/G Agarose or Protein A/G Magrose), or directly using a medium coupled with specific antibodies (e.g. agarose or magnetic beads), and then isolating the antigen-antibody complexes from the complicated protein samples by centrifugation or magnetic force, so as to achieve the purpose of isolation and purification of the target proteins, which can subsequently be used for Western blot or mass spectrometry. Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42 kDa. Protein G is an immunoglobulin binding protein expressed by Streptococcal bacteria type C or G. Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to agarose can be used for immunoprecipitation or antibody purification. Protein A agarose are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, while Protein G agarose beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG2a

This product adopts the self-developed and produced Protein A labelled agarose, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.; This kit provides two elution methods (denaturing elution and acid elution) to elute the target protein, especially the acid elution will not contain the light and heavy chains of the antibody, which can effectively solve the problem of the interference of the antibody's heavy and light chains in the immunoprecipitation and protein immunoblotting experiments.

Characteristics	Description
Product Content	50%(v/v) agarose in specific protective buffer
Beads structure	6% cross-linked agarose
Coupled protein	Protein A
M.W. of Protein	~25 kDa (Protein A)
Binding protein capacity	>1mg mouse antibody per ml beads
Specificity	Antibodies from different species, including mice, humans, rats, goats, sheep
	and cattle
Bead Diameter	30~150 μm
	Elution with acid, or 1x SDS-PAGE loading buffer (reduced)
Elution Methods	Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~
	50kDa) and light chain (~ 25kDa) of the antibody will be denatured and
	released from the agarose beads.
Applications	IP, Co-IP, Protein purification

#### **Product Information**

# Storage and Shipping Conditions

Ship with wet ice; 5×SDS-PAGE loading buffer (reduced) should be stored at -20°C and other at 2-8°C for 12 months.

# Product Components

Component Number	Component	G2215-50T	Storage temperature
G2038	IP lysis buffer	50 mL	2-8°C
G0015	10×TBS	5 mL	2-8°C
G2215-1	SweAgarose Protein A	1 mL	2-8°C
G2215-2	Acid Elution Buffer	10 mL	2-8°C
G2215-3	Neulization Elution Buffer	1 mL	2-8°C
G2013	5x SDS-PAGE loading buffer (reduced)	1 mL	-20°C
Manual			1pc

#### Additional Reagents Required

Product Name	Cat. Number	Spec.
50×Cocktail protease inhibitor	G2006-250UL	250 μL
PBS,1×(Phosphate Buffered Saline)	G4202-500ML	500 mL

#### Assay Protocol/Procedures

#### 1. Preparation of the kit

a) Refer to the table below, prepare the relevant reagents at a ratio of 100-500 μL of lysate per sample;

Step	Solution Required	Volum	Volum
Cell lysis and	IP lysate containing Cocktail	100 ul	500 ul
preparation	protease inhibitor		000 µL
IP	SweAgarose Protein A	4 μL	20 μL
Wash three times	1×TBS	100 μL	500µL
Acid Elution &	Acid Elution Buffer	20 µL	100 µL
Neutralisation	Neutralization Buffer	20 µL	100 µL
Denaturing Elution	1x SDS-PAGE loading buffer (reduced)	20 μL	100 μL

- b) Preparation of IP lysate containing protease inhibitor: Refer to the above table, use 100-200 μL of IP lysate containing protease inhibitor per 0.5-1 million cells for lysis; mix the IP lysate with 50x Cocktail protease inhibitor (G2006-250UL is recommended) at a ratio of 50:1, for example, add 20 μL of 50x Cocktail protease inhibitor into 1 mL of IP lysate, then 1 mL of IP lysate containing protease inhibitor will be obtained; the prepared IP lysate containing the inhibitor should be placed in an ice bath or at 4°C
- c) Note: If the target protein of the immunoprecipitation involves phosphorylation or acetylation modification, phosphatase inhibitor or deacetylase inhibitor should be added (self-provided); IP lysate containing inhibitor should be prepared prior to use, and should not be frozen and retained for subsequent use.
- Preparation of 1×TBS: Dilute 10×TBS buffer with ultra-pure water to 1×TBS. For example, Add 1 mL of 10×TBS buffer to 9 mL of ultra-pure water, which is 1×TBS buffer after mix well.
- e) Preparation of 1x SDS-PAGE loading buffer (reduced): an appropriate amount of 5x SDS-PAGE loading buffer (reduced) was diluted 5 times with ultra-pure water to make 1x SDS-PAGE loading

buffer (reduced); for example, mix 0.2mL of 5x protein buffer (reduced) with 0.8 mL ultra-pure water, which is 1x SDS-PAGE loading buffer (reduced).

#### 2. Antigen sample preparation

Immunoprecipitation or immunoprecipitation experiments should be carried out immediately after samples lysed, if not, the samples can be stored in the refrigerator at  $-20^{\circ}$ C or  $-80^{\circ}$ C, but freezing and thawing may affect protein-protein interactions; all sample lysis steps should be operated in an ice bath or at 4°C in order to minimise the degradation of protein , and a certain amount of the sample should be taken as an Input or a Total after the sample has been prepared for subsequent detection by Western Blot.

For tissue samples:

- a) The tissues should be rinsed in pre-cold PBS (G4202 recommended) to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice.
- b) Add the IP lysate containing protease inhibitor at a ratio of 100-200 µL per 10-20 mg of tissue, or reduce the amount of IP lysate if higher concentrations of protein are required.
- c) Homogenise with a glass homogeniser or handheld homogeniser, or use our self-developed KZ-III-F low-temperature grinder for full grinding.
- d) The homogenate was transferred to a 1.5 mL centrifuge tube, shaken and mixed, and ice-bathed for 30 min, repeat blown with a pipette every 10 min to ensure complete lysis of the tissue cells.
- e) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For adherent cell samples:

- a) If necessary, the cells can be washed 2-3 times with PBS, absorb the residual liquid thoroughly at the last time.
- b) Scrape the cells with a cell scraper or trypsin digest the cells to make them fully suspended, collect them into a 1.5 mL centrifuge tube and centrifuge at 1,000 x g for 5 min at 4°C, discard the supernatant to collect the cell precipitate.
- c) Add 100-200 µL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells, and there should be no obvious cellular precipitation after fully lysed; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- d) After sufficient lysis, centrifuge at 12,000 x g for 5 minutes at 4 ℃, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For suspend cell samples:

- a) Centrifuge at 1,000 x g for 5 min at 4°C to collect the precipitate; if necessary, wash once with PBS, then aspirate the residual liquid and gently vortex to disperse the cells as much as possible.
- b) Add 100-200 µL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- c) Ice bath for 5 min, centrifugation at 12000 g 4 ℃ for 5 min, take the supernatant, that is, the total protein solution, which can be used for subsequent immunoprecipitation or immunoprecipitation experiments and so on.

For bacterial or yeast samples:

a) Take 1 mL of bacterial or yeast solution and centrifuge to remove the supernatant. If necessary,

the cells can be washed once with PBS, absorb the residual liquid thoroughly at the last time. Gently vortex or flick the bottom of the tube to disperse the cells as much as possible.

- b) Add 100-200 µL of IP lysate containing protease inhibitor and blow gently to fully disperse the bacteria or fungi.
- c) Ice bath for 10 min, for better lysis, bacteria and yeast can be digested with lysozyme and wall-breaking enzyme (self-provided) respectively, and then lysed with IP lysate containing protease inhibitors.
- d) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

#### 3. Agarose preparation

- a) Gently resuspend the SweAgarose Protein A beads to form a homogeneous bead dispersion as much as possible, add 20 µl of well-mixed bead dispersion for every 500 µl of sample, take an appropriate amount of agarose beads into a clean EP tube and add 1x TBS to a final volume of 0.5 mL (20 µl of bead dispersion for each sample is used in the following immunoprecipitation steps as an example).
- b) Gently resuspend the beads, centrifuge at  $6,000 \times g$  for 30s at 4 °C, carefully remove the supernatant and repeat the above steps twice.
- c) The beads were resuspended with 1x TBS according to the volume of the initial bead dispersion.

#### 4. Antibody binding to SweAgarose Protein A beads

- a) Antibody preparation: dilute the antibody with 1x TBS to prepare the antibody working solution according to the dilution ratio recommended in the antibody instructions, or prepare the antibody into an antibody working solution with a final concentration of 5-50  $\mu$ g/mL, which can be prepared on ice; optional: use normal IgG of the same antibody species to prepare normal IgG working solution with the same dilution ratio or final concentration of 5-50  $\mu$ g/mL, remove non-specific binding or serve as a negative control. The normal IgG of the same species means that if the antibody used in subsequent immunoprecipitation is mouse IgG, an appropriate amount of normal mouse IgG can be diluted with 1x TBS in this step to reduce the background or as a negative control.
- b) Antibody adsorption: Separate the SweAgarose Protein A beads prepared in step 3 by centrifuge at 6,000 x g for 30s at 4°C, aspirate the supernatant, add 500 µL of antibody working solution or normal IgG working solution, resuspend and then incubate for 15-60 min at room temperature on a turnover mixer.

Note: It is also possible to incubate the SweAgarose Protein A beads prepared in step 3 directly with an appropriate amount of antibody or normal IgG.

c) Beads separation and washing: Separate the incubated beads by centrifuge at 6,000 x g for 30s at 4°C, aspirate the supernatant, add 500 µL of 1×TBS, resuspend the SweAgarose Protein A beads by gently blowing with a pipette, centrifuge at 6,000 x g for 30s at 4°C, remove the supernatant, repeat the washing for three times, and resuspend the beads with 1×TBS in the same amount as the initial volume.

Note: If the beads are agglomerated or flaky during incubation and washing, it is a normal phenomenon and will not affect the experimental results.

#### 5. Immunoprecipitation (IP):

a) Removal of non-specific binding (optional): the SweAgarose Protein A beads prepared in step 4, which bind normal IgG, are incubated with the samples at 4°C for 60 min and separated by

centrifuge at 6,000 x g for 30s at 4  $^{\circ}$ C, and the supernatants are used for the subsequent experiments; the purpose of this step is to remove the proteins that bind non-specifically to normal IgG.

b) Incubation of samples with SweAgarose Protein A beads conjugated with antibody or normal IgG: add SweAgarose Protein A beads conjugated with antibody or normal IgG at the ratio of 20 µl of bead suspension for every 500 µl of protein sample, place on a side-oscillating shaker or a rotary mixer, and incubate for 2 hours at room temperature or overnight at 4°C.

Note 1: If the beads are agglomerated or flaky during incubation and washing, it is a normal phenomenon and will not affect the experimental results.

Note 2: Alternatively, the appropriate amount of antibody or normal IgG can be incubated with the sample for 1-2 hours at room temperature or overnight at 4°C, and then 10-20  $\mu$ L of agarose bead suspension can be added and incubated for 60 min at room temperature.

- c) Centrifuge: after incubation, centrifuge at 6,000 x g for 30s at 4°C to remove the supernatant.
  Note: Retain a portion of the supernatant to test the effect of immunoprecipitation.
- d) Wash: Add 500 µl of 1×TBS, gently blow the resuspended beads with a pipette, centrifuge at 6,000 x g for 30s at 4°C to remove the supernatant and repeat the wash three times.
  Note: You can also assay the OD280 of the washing buffer to determine whether the washing is complete, if the OD280 is more than 0.05, the number of washing times should be increased appropriately.

#### 6. Elution

Depending on the characteristics of the target protein and the requirements of subsequent experiments, one of the following methods can be selected for elution.

a) **Denaturing elution method:** Samples eluted by this method are suitable for SDS-PAGE. Add 25  $\mu$ L of 1× Protein Sampling Buffer (Reduced) to the tube after centrifuge, heat at 95°C for 5 min, and then centeifuge to collect the supernatant for Western blot detection.

b) Acid Elution: The sample eluted by this method retains its original biological activity and can be used for post-functional analysis. Add 20  $\mu$ L of Acid Elution Buffer to the tube after centrifuge, mix well and incubate at room temperature for 10 min, then centrifuge to collect the supernatant into a new EP tube, and immediately add 2  $\mu$ L of Neulization Elution Buffer to adjust the pH of the eluted product to neutral, which can be used for post-functional analysis.

Note: The user can adjust the amount of elution buffer added according to the desired volume.

#### Note

- 1. Do not store SweAgarose Protein A in the kit at -20°C or freeze and thaw repeatedly, otherwise it will affect the performance of magnetic beads and cause unnecessary experimental errors.
- 2. Please read these instructions carefully before performing the immunoprecipitation procedure.
- 3. If the immunoprecipitated target protein involves phosphorylation or acetylation modification, appropriate phosphatase inhibitors and deacetylase inhibitors should be provided.
- 4. Maintain beads at pH 6-8, avoid high speed centrifugation, drying or freezing, which may cause agglomeration of the beads.
- 5. The beads should be mixed thoroughly before use, the mixing operation should be gentle and should not be subjected to violent swirling and shaking to avoid denaturation of the antibody.
- 6. Positive and negative controls (SweAgarose Mouse IgG) are recommended in immunoprecipitation.
- 7. Protein samples should be purified as soon as possible after collection and always placed at 4°C or in

an ice bath to slow down protein degradation or denaturation.

- 8. Agarose beads may gather when use with acid elution, which is a normal phenomenon and does not affect the normal use of magnetic beads.
- 9. This product is restricted to scientific research use by professionals, and is not to be used for clinical diagnosis or treatment, food or medicine, or stored in an ordinary residence.
- 10. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
- 11. The binding capacity of Protein A and Protein G to antibodies of different generic sources and subtypes is shown in Table I.

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
	lgG4	++++	++++
Human	IgA	++	-
	lgD	++	-
	IgE	++	-
	lgM	++	-
Mouse	lgG1	+	++++
	lgG2a	++++	++++
	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

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Total Ig	Protein A	Protein G
Total Ig	THOLEMIA	Trotem o
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

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++++: Strong Bing

- ++~++: Medium Binding
- +: Weak Binding
- +/-: Weak or No Binding
- -:No Binding



# Servicebio<sup>®</sup> Immunoprecipitation Kit (Protein G Magnetic Beads)

# Cat. #:G2210

#### **Product Information**

Product Name	Cat. No.	Spec.
Immunoprecipitation Kit (Protein G Magnetic Beads)	G2210-50T	50 T

## Product Description/Introduction

IP or Co-IP is a common experimental technique for studying protein or protein-protein interactions (PPIs) by using specific antibodies and a medium that binds antibodies (e.g. Protein A/G Agarose or Protein A/G Magrose), or directly using a medium coupled with specific antibodies (e.g. agarose gels or magnetic beads), and then isolating the antigen-antibody complexes from the complicated protein samples by centrifugation or magnetic force, so as to achieve the purpose of isolation and purification of the target proteins, which can subsequently be used for Western blot or mass spectrometry. Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42 kDa. Protein G is an immunoglobulin binding protein expressed by Streptococcal bacteria type C or G. Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to magnetic beads can be used for immunoprecipitation or antibody purification. Protein A magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, while Protein G magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c polyclonal antibodies. (See schedule I for specific information)

This product adopts the self-developed and produced Protein A protein-labelled magnetic beads, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.; This kit provides two elution methods (denaturing elution and acid elution) to elute the target protein, especially the acid elution will not contain the light and heavy chains of the antibody, which can effectively solve the problem of the interference of the antibody's heavy and light chains in the immunoprecipitation and protein immunoblotting experiments.

Characteristics	Description	
Product Content	50mg/ml magnetic beads in specific protective buffer	
Magnetization	Superparamagnetism	
Coupled protein	Protein G	
M.W. of Protein	~25 kDa (Protein G)	
Binding protein capacity	>1mg mouse antibody per ml beads	
Specificity	Antibodies from different species, including mice, humans, rats, goats, sheep and cattle	
Bead Diameter	30~150 μm	
Elution Methods	Elution with acid, or 1x SDS-PAGE loading buffer (reduced) Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~ 50kDa) and light chain (~ 25kDa) of the antibody will be denatured and released	

#### **Product Information**

	from the magnetic beads.
Applications	IP, Co-IP, Protein purification

## **Storage and Shipping Conditions**

Ship with wet ice;  $5 \times SDS$ -PAGE loading buffer (reduced) should be stored at-20 °C and other at 2-8°C for 12 months.

# **Product Components**

Component Number	Component	G2210-50T	Storage temperature
G2038	IP lysis buffer	50 mL	2-8°C
G0015	10×TBS	5 mL	2-8°C
G2210-1	SweMagrose Protein G	1 mL	2-8°C
G2209-2	Acid Elution Buffer	10 mL	2-8°C
G2209-3	Neulization Elution Buffer	1 mL	2-8°C
G2013	5x SDS-PAGE loading buffer (reduced)	1 mL	-20°C
Manual			1 pc

## **Additional Reagents Required**

Product Name	Cat. Number	Spec.
50×Cocktail protease inhibitor	G2006-250UL	250 μL
PBS,1×(Phosphate Buffered Saline)	G4202-500ML	500 mL

#### Assay Protocol/Procedures

#### 1. Preparation of the kit

 Refer to the table below, prepare the relevant reagents at a ratio of 100-500 μL of lysate per sample.

Step	Solution Required	Volum	Volum
Cell lysis and preparation	IP lysate containing Cocktail protease inhibitor	100 µL	500 μL
IP	SweMagrose Protein G	4 μL	20 μL
Wash three times	1×TBS	100 μL	500µL
Acid Elution &	Acid Elution Buffer	20 µL	100 μL
Neutralisation	Neutralization Buffer	20 µL	100 μL
Denaturing Elution	1×SDS-PAGE loading buffer (reduced)	20 μL	100 μL

- b) Preparation of IP lysate containing protease inhibitor: Refer to the above table, use 100-200 µL of IP lysate containing protease inhibitor per 0.5-1 million cells for lysis; mix the IP lysate with 50x Cocktail protease inhibitor (G2006-250UL is recommended) at a ratio of 50:1, for example, add 20 µL of 50x Cocktail protease inhibitor into 1 mL of IP lysate, then 1 mL of IP lysate containing protease inhibitor will be obtained; the prepared IP lysate containing the inhibitor should be placed in an ice bath or at 4°C.
- c) Note: If the target protein of the immunoprecipitation involves phosphorylation or acetylation modification, phosphatase inhibitor or deacetylase inhibitor should be added (self-provided); IP lysate containing inhibitor should be prepared prior to use, and should not be frozen and retained for subsequent use.
- Preparation of 1×TBS: Dilute 10×TBS buffer with ultra-pure water to 1×TBS. For example, Add 1
  mL of 10×TBS buffer to 9 mL of ultra-pure water, which is 1×TBS buffer after mix well.

e) Preparation of 1x SDS-PAGE loading buffer (reduced): an appropriate amount of 5x SDS-PAGE loading buffer (reduced) was diluted 5 times with ultra-pure water to make 1x SDS-PAGE loading buffer (reduced); for example, mix 0.2mL of 5x protein buffer (reduced) with 0.8 mL ultra-pure water, which is 1x SDS-PAGE loading buffer (reduced).

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#### 2. Antigen sample preparation

Immunoprecipitation or immunoprecipitation experiments should be carried out immediately after samples lysed, if not, the samples can be stored in the refrigerator at  $-20^{\circ}$ C or  $-80^{\circ}$ C, but freezing and thawing may affect protein-protein interactions; all sample lysis steps should be operated in an ice bath or at 4°C in order to minimise the degradation of protein , and a certain amount of the sample should be taken as an Input or a Total after the sample has been prepared for subsequent detection by Western Blot.

For tissue samples:

- a) The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice.
- b) Add the IP lysate containing protease inhibitor at a ratio of 100-200 µL per 10-20 mg of tissue, or reduce the amount of IP lysate if higher concentrations of protein are required.
- Homogenise with a glass homogeniser or handheld homogeniser, or use our self-developed KZ-III-F low-temperature grinder for full grinding.
- d) The homogenate was transferred to a 1.5 mL centrifuge tube, shaken and mixed, and ice-bathed for 30 min, repeat blown with a pipette every 10 min to ensure complete lysis of the tissue cells.
- e) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For adherent cell samples:

- a) If necessary, the cells can be washed 2-3 times with PBS, absorb the residual liquid thoroughly at the last time.
- b) Scrape the cells with a cell scraper or trypsin digest the cells to make them fully suspended, collect them into a 1.5 mL centrifuge tube and centrifuge at 1,000 x g for 5 min at 4°C, discard the supernatant to collect the cell precipitate.
- c) Add 100-200 μL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells, and there should be no obvious cellular precipitation after fully lysed; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- After sufficient lysis, centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For suspend cell samples:

- a) Centrifuge at 1000 g for 5 min at 4°C to collect the precipitate; if necessary, wash once with PBS, then aspirate the residual liquid and gently vortex or flick the bottom of the tube to disperse the cells as much as possible.
- b) Add 100-200 μL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- c) Ice bath for 5 min, centrifugation at 12000 g 4 ℃ for 5 min, take the supernatant, that is, the total protein solution, which can be used for subsequent immunoprecipitation or

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immunoprecipitation experiments and so on.

For bacterial or yeast samples:

- a) Take 1 mL of bacterial or yeast solution and centrifuge to remove the supernatant. If necessary, the cells can be washed once with PBS, absorb the residual liquid thoroughly at the last time.
  Gently vortex or flick the bottom of the tube to disperse the cells as much as possible.
- b) Add 100-200 µL of IP lysate containing protease inhibitor and blow gently to fully disperse the bacteria or fungi.
- c) Ice bath for 10 min, for better lysis, bacteria and yeast can be digested with lysozyme and wall-breaking enzyme (self-provided) respectively, and then lysed with IP lysate containing protease inhibitors.
- d) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

#### 3. Magnetic bead preparation

- a) Gently resuspend the SweMagrose Protein G magnetic beads to form a homogeneous bead dispersion as much as possible, add 20 μl of well-mixed bead dispersion for every 500 μl of sample, take an appropriate amount of magnetic beads into a clean EP tube and add 1x TBS to a final volume of 0.5 mL (20 μl of bead dispersion for each sample is used in the following immunoprecipitation steps as an example).
- b) Gently resuspend the magnetic beads, place on a magnetic separation stand for 30 s, carefully remove the supernatant and repeat the above steps twice.
- c) The magnetic beads were resuspended with 1x TBS according to the volume of the initial bead dispersion.

#### 4. Antibody binding to SweMagrose Protein G magnetic beads

- a) Antibody preparation: dilute the antibody with 1x TBS to prepare the antibody working solution according to the dilution ratio recommended in the antibody instructions, or prepare the antibody into an antibody working solution with a final concentration of 5-50  $\mu$ g/mL, which can be prepared on ice; **optional:** use normal IgG of the same antibody species to prepare normal IgG working solution with the same dilution ratio or final concentration of 5-50  $\mu$ g/mL, remove non-specific binding or serve as a negative control. The normal IgG of the same species means that if the antibody used in subsequent immunoprecipitation is mouse IgG, an appropriate amount of normal mouse IgG can be diluted with 1x TBS in this step to reduce the background or as a negative control.
- b) Antibody adsorption: Separate the SweMagrose Protein G magnetic beads prepared in step 3 by magnetic, aspirate the supernatant, add 500 µL of antibody working solution or normal IgG working solution, resuspend and then incubate for 15-60 min at room temperature on a turnover mixer.

# Note: It is also possible to incubate the SweMagrose Protein G magnetic beads prepared in step 3 directly with an appropriate amount of antibody or normal IgG.

c) Magnetic separation and washing: Separate the incubated beads by magnetic, aspirate the supernatant, add 500 µL of 1 × TBS, resuspend the SweMagrose Protein G beads by gently blowing with a pipette, place on a magnetic separation rack for 10 s, remove the supernatant, repeat the washing for three times, and resuspend the beads with 1×TBS in the same amount as the initial volume.

Note: If the beads are agglomerated or flaky during incubation and washing, it is a normal

#### phenomenon and will not affect the experimental results.

#### 5. Immunoprecipitation (IP) :

- a) Removal of non-specific binding (optional): the SweMagrose Protein G beads prepared in step 4, which bind normal IgG, are incubated with the samples at 4°C for 60 min and separated by magnetic suction, and the supernatants are used for the subsequent experiments; the purpose of this step is to remove the proteins that bind non-specifically to normal IgG.
- b) Incubation of samples with SweMagrose Protein G beads conjugated with antibody or normal IgG: add SweMagrose Protein G beads conjugated with antibody or normal IgG at the ratio of 20 μl of bead suspension for every 500 μl of protein sample, place on a side-oscillating shaker or a rotary mixer, and incubate for 2 hours at room temperature or overnight at 4°C.

Note 1: If the beads are agglomerated or flaky during incubation and washing, it is a normal phenomenon and will not affect the experimental results.

Note 2: Alternatively, the appropriate amount of antibody or normal IgG can be incubated with the sample for 1-2 hours at room temperature or overnight at 4°C, and then 10-20  $\mu$ L of magnetic bead suspension can be added and incubated for 60 min at room temperature.

c) Magnetic separation: after incubation, place on a magnetic separation stand for 10 seconds to remove the supernatant.

#### Note: Retain a portion of the supernatant to test the effect of immunoprecipitation.

d) Wash: Add 500 µl of 1 × TBS, gently blow the resuspended beads with a pipette, place on a magnetic separator for 10 seconds to remove the supernatant and repeat the wash three times..
 Note: You can also test the OD280 of the washing solution to determine whether the washing is complete, if the OD280 is more than 0.05, the number of washing times should be increased appropriately.

#### 6. Elution

Depending on the characteristics of the target protein and the requirements of subsequent experiments, one of the following methods can be selected for elution.

- a) **Denaturing elution method:** Samples eluted by this method are suitable for SDS-PAGE. Remove the EP tube from the magnetic separator, add 25  $\mu$ L of 1× Protein Sampling Buffer (Reduced) to the tube, heat at 95 °C for 5 min, and then perform a magnetic separation to collect the supernatant for Western blot detection.
- b) Acid Elution: The sample eluted by this method retains its original biological activity and can be used for post-functional analysis. Remove the EP tube from the magnetic separation rack, add 20 μL of Acid Elution Buffer to the tube, mix well and incubate at room temperature for 10 min, then perform a magnetic suction separation, collect the supernatant into a new EP tube, and immediately add 2 μL of Neulization Elution Buffer to adjust the pH of the eluted product to neutral, which can be used for post-functional analysis.

Note: The user can adjust the amount of elution buffer added according to the desired volume.

#### Note

- 1. Do not store SweMagrose Protein A in the kit at -20°C or freeze and thaw repeatedly, otherwise it will affect the performance of magnetic beads and cause unnecessary experimental errors.
- 2. Please read these instructions carefully before performing the immunoprecipitation procedure.
- 3. Magnetic separation frame for magnetic separation need self-provided
- 4. If the immunoprecipitated target protein involves phosphorylation or acetylation modification,



appropriate phosphatase inhibitors and deacetylase inhibitors should be provided.

- 5. Maintain beads at pH 6-8, avoid high speed centrifugation, drying or freezing; do not expose beads to magnetic fields for long periods of time which may cause agglomeration of the beads.
- 6. The beads should be mixed thoroughly before use, the mixing operation should be gentle and should not be subjected to violent swirling and shaking to avoid denaturation of the antibody.
- 7. Positive and negative controls (SweMagrose Mouse IgG) are recommended in immunoprecipitation.
- 8. Protein samples should be purified as soon as possible after collection and always placed at 4°C or in an ice bath to slow down protein degradation or denaturation
- 9. Magnetic beads may gather when use with acid elution, which is a normal phenomenon and does not affect the normal use of magnetic beads.
- 10. 10. This product is restricted to scientific research use by professionals, and is not to be used for clinical diagnosis or treatment, food or medicine, or stored in an ordinary residence.
- 11. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
- 12. The binding capacity of Protein A and Protein G to antibodies of different generic sources and subtypes is shown in Table I.

Species	lg	Protein A	Protein G
	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
Lluraan	lgG4	++++	++++
Human	IgA	++	-
	IgD	++	-
	IgE	++	-
	lgM	++	-
	lgG1	+	++++
	lgG2a	++++	++++
Mouse	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	lgM	+/-	-

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding

Table 1



# Servicebio<sup>®</sup> Immunoprecipitation Kit (Protein A Magnetic Beads)

# Cat. #:G2209

#### **Product Information**

Product Name	Cat. No.	Spec.
Immunoprecipitation Kit (Protein A Magnetic Beads)	G2209-50T	50T

#### Product Description/Introduction

IP or Co-IP is a common experimental technique for studying protein or protein-protein interactions (PPIs) by using specific antibodies and a medium that binds antibodies (e.g. Protein A/G Agarose or Protein A/G Magrose), or directly using a medium coupled with specific antibodies (e.g. agarose gels or magnetic beads), and then isolating the antigen-antibody complexes from the complicated protein samples by centrifugation or magnetic force, so as to achieve the purpose of isolation and purification of the target proteins, which can subsequently be used for Western blot or mass spectrometry. Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42 kDa. Protein G is an immunoglobulin binding protein expressed by Streptococcal bacteria type C or G. Protein A and Protein G are functionally similar and can bind specifically to mammalian immunoglobulin (Ig). Recombinant Protein A and G with appropriate modifications bound to magnetic beads can be used for immunoprecipitation or antibody purification. Protein A magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, while Protein G magnetic beads are suitable for the immunoprecipitation of human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c polyclonal antibodies. (See Table I for specific information)

This product adopts the self-developed and produced Protein A protein-labelled magnetic beads, compared with similar products in the market, this product binds antibodies more efficiently and has a lower non-specific binding rate, together with the optimized buffer, it can be convenient and efficient for immunoprecipitation experiments; it can be widely used in the isolation and purification of the target proteins in samples such as cell lysates, cellular secretion supernatants, serum, ascites, etc.; This kit provides two elution methods (denaturing elution and acid elution) to elute the target protein, especially the acid elution will not contain the light and heavy chains of the antibody, which can effectively solve the problem of the interference of the antibody's heavy and light chains in the immunoprecipitation and protein immunoblotting experiments.

Characteristics	Description	
Product Content	50mg/ml magnetic beads in specific protective buffer	
Magnetization	Superparamagnetism	
Coupled protein	Protein A	
M.W. of Protein	~25 kDa(Protein A)	
Binding protein capacity	>1mg mouse antibody per ml beads	
Specificity	Antibodies from different species, including mice, humans, rats, goats, sheep and cattle	
Bead Diameter 30~150 μm		
Elution Methods	Elution with acid, or 1x SDS-PAGE loading buffer (reduced) Note: If eluted with 1x SDS-PAGE loading buffer (reduced), the heavy chain (~ 50kDa) and light chain (~ 25kDa) of the antibody will be denatured and	

#### **Product Information**

	released from the magnetic beads.	
Applications	IP, Co-IP, Protein purification	

# **Storage and Shipping Conditions**

Ship with wet ice; 5×SDS-PAGE loading buffer (reduced) should be stored at -20°C and other at 2-8°C for 12 months.

# **Product Components**

Component Number	Component	G2209-50T	Storage temperature
G2038	IP lysis buffer	50 mL	2-8°C
G0015	10×TBS	5 mL	2-8°C
G2209-1	SweMagrose Protein A	1 mL	2-8°C
G2209-2	Acid Elution Buffer	10 mL	2-8°C
G2209-3	Neulization Elution Buffer	1 mL	2-8°C
G2013	5x SDS-PAGE loading buffer (reduced)	1 mL	-20°C
Manual			1 pc

## **Additional Reagents Required**

Product Name	Cat. Number	Spec.
50×Cocktail protease inhibitor	G2006-250UL	250 μL
PBS,1×(Phosphate Buffered Saline)	G4202-500ML	500 mL

#### Assay Protocol/Procedures

#### 1. Preparation of the kit

a) Refer to the table below, prepare the relevant reagents at a ratio of 100-500  $\mu$ L of lysate per sample;

Step	Solution Required	Volum	Volum
Cell lysis and	IP lysate containing Cocktail	100 ul	500 ul
preparation	protease inhibitor	100 με	500 με
IP	SweMagrose Protein A	4 μL	20 μL
Wash three times	1×TBS	100 μL	500µL
Acid Elution &	Acid Elution Buffer	20 µL	100 µL
Neutralisation	Neutralization Buffer	20 µL	100 μL
Denaturing Elution	1x SDS-PAGE loading buffer (reduced)	20 µL	100 μL

- b) Preparation of IP lysate containing protease inhibitor: Refer to the above table, use 100-200 μL of IP lysate containing protease inhibitor per 0.5-1 million cells for lysis; mix the IP lysate with 50x Cocktail protease inhibitor (G2006-250UL is recommended) at a ratio of 50:1, for example, add 20 μL of 50x Cocktail protease inhibitor into 1 mL of IP lysate, then 1 mL of IP lysate containing protease inhibitor will be obtained; the prepared IP lysate containing the inhibitor should be placed in an ice bath or at 4°C.
- c) Note: If the target protein of the immunoprecipitation involves phosphorylation or acetylation modification, phosphatase inhibitor or deacetylase inhibitor should be added (self-provided); IP lysate containing inhibitor should be prepared prior to use, and should not be frozen and retained for subsequent use.
- d) Preparation of 1×TBS: Dilute 10×TBS buffer with ultra-pure water to 1×TBS. For example, Add 1 mL of 10×TBS buffer to 9 mL of ultra-pure water, which is 1×TBS buffer after mix well.

Preparation of 1x SDS-PAGE loading buffer (reduced): an appropriate amount of 5x SDS-PAGE loading buffer (reduced) was diluted 5 times with ultra-pure water to make 1x SDS-PAGE loading buffer (reduced); for example, mix 0.2mL of 5x protein buffer (reduced) with 0.8 mL ultra-pure water, which is 1x SDS-PAGE loading buffer (reduced).

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#### 2. Antigen sample preparation

e)

Immunoprecipitation or immunoprecipitation experiments should be carried out immediately after samples lysed, if not, the samples can be stored in the refrigerator at  $-20^{\circ}$ C or  $-80^{\circ}$ C, but freezing and thawing may affect protein-protein interactions; all sample lysis steps should be operated in an ice bath or at 4°C in order to minimise the degradation of protein , and a certain amount of the sample should be taken as an Input or a Total after the sample has been prepared for subsequent detection by Western Blot.

For tissue samples:

- a) The tissues should be rinsed in pre-cold PBS to remove excess blood thoroughly. Weigh and mince into small pieces in homogenizer on ice.
- b) Add the IP lysate containing protease inhibitor at a ratio of 100-200 µL per 10-20 mg of tissue, or reduce the amount of IP lysate if higher concentrations of protein are required.
- Homogenise with a glass homogeniser or handheld homogeniser, or use our self-developed KZ-III-F low-temperature grinder for full grinding.
- d) The homogenate was transferred to a 1.5 mL centrifuge tube, shaken and mixed, and ice-bathed for 30 min, repeat blown with a pipette every 10 min to ensure complete lysis of the tissue cells.
- e) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For adherent cell samples:

- a) If necessary, the cells can be washed 2-3 times with PBS, absorb the residual liquid thoroughly at the last time.
- b) Scrape the cells with a cell scraper or trypsin digest the cells to make them fully suspended, collect them into a 1.5 mL centrifuge tube and centrifuge at 1,000 x g for 5 min at 4°C, discard the supernatant to collect the cell precipitate.
- c) Add 100-200 μL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells, and there should be no obvious cellular precipitation after fully lysed; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- After sufficient lysis, centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

For suspend cell samples:

- a) Centrifuge at 1000 g for 5 min at 4°C to collect the precipitate; if necessary, wash once with PBS, then aspirate the residual liquid and gently vortex or flick the bottom of the tube to disperse the cells as much as possible.
- b) Add 100-200 μL of IP lysate containing protease inhibitor per 0.5-1 million cells (equivalent to one well of a 6-well plate); blow appropriately and ice bath for 3-5 min to fully lysed the cells; if the amount of cells is larger, it is recommended that the cells be divided into 0.5-1 million cells/tube to fully lysis.
- c) Ice bath for 5 min, centrifugation at 12000 g 4℃ for 5 min, take the supernatant, that is, the total protein solution, which can be used for subsequent immunoprecipitation or immunoprecipitation

experiments and so on.

For bacterial or yeast samples:

 a) Take 1 mL of bacterial or yeast solution and centrifuge to remove the supernatant. If necessary, the cells can be washed once with PBS, absorb the residual liquid thoroughly at the last time. Gently vortex or flick the bottom of the tube to disperse the cells as much as possible.

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- b) Add 100-200 µL of IP lysate containing protease inhibitor and blow gently to fully disperse the bacteria or fungi.
- c) Ice bath for 10 min, for better lysis, bacteria and yeast can be digested with lysozyme and wall-breaking enzyme (self-provided) respectively, and then lysed with IP lysate containing protease inhibitors.
- d) Centrifuge at 12,000 x g for 5 minutes at 4°C, discard the precipitate and aspirate supernatant for subsequent immunoprecipitation or immunoprecipitation experiments.

#### 3. Magnetic bead preparation

- a) Gently resuspend the SweMagrose Protein A magnetic beads to form a homogeneous bead dispersion as much as possible, add 20 μl of well-mixed bead dispersion for every 500 μl of sample, take an appropriate amount of magnetic beads into a clean EP tube and add 1x TBS to a final volume of 0.5 mL (20 μl of bead dispersion for each sample is used in the following immunoprecipitation steps as an example).
- b) Gently resuspend the magnetic beads, place on a magnetic separation stand for 30 s, carefully remove the supernatant and repeat the above steps twice.
- c) The magnetic beads were resuspended with 1x TBS according to the volume of the initial bead dispersion.

#### 4. Antibody binding to SweMagrose Protein A magnetic beads

- a) Antibody preparation: dilute the antibody with 1x TBS to prepare the antibody working solution according to the dilution ratio recommended in the antibody instructions, or prepare the antibody into an antibody working solution with a final concentration of 5-50  $\mu$ g/mL, which can be prepared on ice; **optional:** use normal IgG of the same antibody species to prepare normal IgG working solution with the same dilution ratio or final concentration of 5-50  $\mu$ g/mL, remove non-specific binding or serve as a negative control. The normal IgG of the same species means that if the antibody used in subsequent immunoprecipitation is mouse IgG, an appropriate amount of normal mouse IgG can be diluted with 1x TBS in this step to reduce the background or as a negative control.
- b) Antibody adsorption: Separate the SweMagrose Protein A magnetic beads prepared in step 3 by magnetic, aspirate the supernatant, add 500 µL of antibody working solution or normal IgG working solution, resuspend and then incubate for 15-60 min at room temperature on a turnover mixer.

# Note: It is also possible to incubate the SweMagrose Protein A magnetic beads prepared in step 3 directly with an appropriate amount of antibody or normal IgG.

c) Magnetic separation and washing: Separate the incubated beads by magnetic, aspirate the supernatant, add 500 µL of 1 × TBS, resuspend the SweMagrose Protein A beads by gently blowing with a pipette, place on a magnetic separation rack for 10 s, remove the supernatant, repeat the washing for three times, and resuspend the beads with 1×TBS in the same amount as the initial volume.

Note: If the beads are agglomerated or flaky during incubation and washing, it is a normal

#### phenomenon and will not affect the experimental results.

#### 5. Immunoprecipitation (IP) :

- a) Removal of non-specific binding (optional): the SweMagrose Protein A beads prepared in step 4, which bind normal IgG, are incubated with the samples at 4°C for 60 min and separated by magnetic suction, and the supernatants are used for the subsequent experiments; the purpose of this step is to remove the proteins that bind non-specifically to normal IgG.
- b) Incubation of samples with SweMagrose Protein A beads conjugated with antibody or normal IgG: add SweMagrose Protein A beads conjugated with antibody or normal IgG at the ratio of 20 μl of bead suspension for every 500 μl of protein sample, place on a side-oscillating shaker or a rotary mixer, and incubate for 2 hours at room temperature or overnight at 4°C.

Note 1: If the beads are agglomerated or flaky during incubation and washing, it is a normal phenomenon and will not affect the experimental results.

Note 2: Alternatively, the appropriate amount of antibody or normal IgG can be incubated with the sample for 1-2 hours at room temperature or overnight at 4°C, and then 10-20  $\mu$ L of magnetic bead suspension can be added and incubated for 60 min at room temperature.

c) Magnetic separation: after incubation, place on a magnetic separation stand for 10 seconds to remove the supernatant.

#### Note: Retain a portion of the supernatant to test the effect of immunoprecipitation.

d) Wash: Add 500 µl of 1 × TBS, gently blow the resuspended beads with a pipette, place on a magnetic separator for 10 seconds to remove the supernatant and repeat the wash three times.
 Note: You can also test the OD280 of the washing solution to determine whether the washing is complete, if the OD280 is more than 0.05, the number of washing times should be increased appropriately.

#### 6. Elution

Depending on the characteristics of the target protein and the requirements of subsequent experiments, one of the following methods can be selected for elution.

a) **Denaturing elution method:** Samples eluted by this method are suitable for SDS-PAGE. Remove the EP tube from the magnetic separator, add 25  $\mu$ L of 1 × Protein Sampling Buffer (Reduced) to the tube, heat at 95°C for 5 min, and then perform a magnetic separation to collect the supernatant for Western blot detection.

b) Acid Elution: The sample eluted by this method retains its original biological activity and can be used for post-functional analysis. Remove the EP tube from the magnetic separation rack, add 20  $\mu$ L of Acid Elution Buffer to the tube, mix well and incubate at room temperature for 10 min, then perform a magnetic suction separation, collect the supernatant into a new EP tube, and immediately add 2  $\mu$ L of Neulization Elution Buffer to adjust the pH of the eluted product to neutral, which can be used for post-functional analysis.

Note: The user can adjust the amount of elution buffer added according to the desired volume.

#### Note

- 1. Do not store SweMagrose Protein A in the kit at -20°C or freeze and thaw repeatedly, otherwise it will affect the performance of magnetic beads and cause unnecessary experimental errors.
- 2. Please read these instructions carefully before performing the immunoprecipitation procedure.
- 3. Magnetic separation frame for magnetic separation need self-provided
- 4. If the immunoprecipitated target protein involves phosphorylation or acetylation modification,



appropriate phosphatase inhibitors and deacetylase inhibitors should be provided.

- 5. Maintain beads at pH 6-8, avoid high speed centrifugation, drying or freezing; do not expose beads to magnetic fields for long periods of time which may cause agglomeration of the beads.
- 6. The beads should be mixed thoroughly before use, the mixing operation should be gentle and should not be subjected to violent swirling and shaking to avoid denaturation of the antibody.
- 7. Positive and negative controls (SweMagrose Mouse IgG) are recommended in immunoprecipitation.
- 8. Protein samples should be purified as soon as possible after collection and always placed at 4°C or in an ice bath to slow down protein degradation or denaturation
- 9. Magnetic beads may gather when use with acid elution, which is a normal phenomenon and does not affect the normal use of magnetic beads.
- 10. This product is restricted to scientific research use by professionals, and is not to be used for clinical diagnosis or treatment, food or medicine, or stored in an ordinary residence.
- 11. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
- 12. The binding capacity of Protein A and Protein G to antibodies of different generic sources and subtypes is shown in Table I.

1			
Species	lg	Protein A	Protein G
Human	lgG1	++++	++++
	lgG2	++++	++++
	lgG3	-	++++
	lgG4	++++	++++
	IgA	++	-
	IgD	++	-
	IgE	++	-
	lgM	++	-
Mouse	lgG1	+	++++
	lgG2a	++++	++++
	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG2c	+	++
	IgM	+/-	-

Total Ig	Protein A	Protein G
Human	++++	++++
Mouse	+++	+++
Rat	+/-	++
Rabbit	++++	+++
Goat	-	++
Chicken	-	+
Cow	++	++++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Pig	+++	+++
Sheep	+/-	++

++++: Strong Bing

++~++: Medium Binding

+: Weak Binding

+/-: Weak or No Binding

-:No Binding

Table 1

# 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 Волоград (844)278-03-48 Волоград (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 Краснодар (861)203-40-90 Красноярск (391)204-63-61 Курсак (4712)77-13-04 Куртан (3522)50-90-47 Липецк (4742)52-20-81

Россия +7(495)268-04-70

Магнитогорск (3519)55-03-13 Москва (495)268-04-70 Мурманск (8152)59-64-93 Набережные Челны (8552)20-53-41 Нижний Новгород (831)429-08-12 Новокузнецк (3843)20-46-81 Ноябрьск (3496)41-32-12 Новосибирск (383)227-86-73 Омск (3812)21-46-40 Орел (4862)44-53-42 Оренбург (3522)37-68-04 Пенза (8412)22-31-16 Петрозаводск (8142)55-98-37 Псков (8112)59-10-37 Пермь (342)205-81-47

Казахстан +7(7172)727-132

Ростов-на-Дону (863)308-18-15 Рязань (4912)46-61-64 Самара (846)206-03-16 Санкт-Петербург (812)309-46-40 Саратов (845)249-38-78 Севастополь (8692)22-31-93 Саранск (8342)22-96-24 Симферополь (3652)67-13-56 Смоленск (4812)29-41-54 Сочи (862)225-72-31 Ставрополь (8652)20-65-13 Сургут (3462)77-98-35 Сыктывкар (8212)25-95-17 Тамбов (4752)50-40-97 Тверь (4822)63-31-35

Киргизия +996(312)96-26-47

Томск (3822)98-41-53 Тула (4872)33-79-87 Тюмень (3452)66-21-18 Ульяновск (8422)24-23-59 Улан-Удэ (3012)59-97-51 Уфа (347)229-48-12 Хабаровск (4212)92-98-04 Чебоксары (8352)28-53-07 Челябинск (351)202-03-61 Череповец (8202)49-02-64 Чита (3022)38-34-83 Якутск (4112)23-90-97 Ярославль (4852)69<u>-52-93</u>

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