Protein A/G beads

Introduce

Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42 kDa, which can specifically bind to the Fc region of mammalian immunoglobulin (Ig) and to the Fab region of the human VH3 family. Protein G is an immunoglobulin-binding protein expressed by Streptococcal bacteria that specifically binds to the Immunoglobulin (Ig) Fc region of mammals. This product is a modified recombinant Protein A (25 kDa) and Protein G (25 kDa), covalently coupled with nanoscale amino beads, and only retains the amino acid sequence associated with Fc terminal binding such as IgG, removing the sequence that may lead to non-specific binding except the binding site, which can effectively reduce non-specific binding.

Protein A beads can specifically bind to corresponding antibodies, such as human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b and rabbit IgG, etc., each Protein A molecule can bind 5 IgG molecules, and Protein G beads are suitable for immunoprecipitation human IgG1, IgG2, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c, as well as rabbit, goat polyclonal antibodies, etc., each Protein G molecule can bind to 3 IgG molecules. The product is mainly used for immunoprecipitation (IP), co-precipitation (Co-IP) or chromatin immunoprecipitation (Ch-IP), as well as the purification of antibodies in samples such as serum, cell culture supernatant or ascites. The binding capacity of common immunoglobulin subclasses and the total binding capacity of different species are shown in the following table (Table 1).

This product is configured with Protein A beads and Protein G beads in a ratio of 1:1, which has a variety of significant advantages. First, high content and binding specificity of the binding antibody can be achieved. Compared with traditional Protein A/G agarose gels, this product has a smaller pore size, is not prone to non-specific adsorption, and has a high binding amount. The 1 ml magnetic bead suspension of this product contains about 10 mg of magnetic beads, not less than 0.6 mg of recombinant Protein A/G and can bind to not less than 0.7 mg of Human IgG. The specific maximum binding amount is related to the type of antibody and the target protein. For experiments, efficient immunoprecipitation experiments are typically performed using 10-20 µl of Protein A/G bead suspension for 500 µl of samples. Second, ultra-rapid binding of antibodies or antibody

complexes can be achieved. Protein A/G beads (~200 nm) facilitate fast and effective binding of beads to antibodies or antibody complexes due to their large specific surface area. The adsorption process of the antibody or its complex can usually be completed within 10 min, 30 min Complete the immunoprecipitation operation of the protein of interest. Shortening the operation time can effectively avoid the degradation or denaturation of the protein of interest during long-term operation, and fully ensure the activity of the protein of interest. Due to the magnetic separation, IP and Co-IP can save 40% of the time compared to agarose gels per time. Finally, a variety of methods can be used to elute. Depending on factors such as the structure, biological function and design requirements of the protein of interest, a variety of eluents such as acidic solution, SDS-PAGE loading buffer or competitive peptides can be used for elutation. (See Table 2 for specific product parameters).

Species	Subclass	Protein A binding	Protein G binding
Human	lgA	++	-
	lgD	++	-
	IgE	++	-
	lgG1	++++	++++
	lgG2	++++	++++
	IgG3	-	++++
	lgG4	++++	++++
	lgM	++	-
Mouse	IgG1	+	++++
	lgG2a	++++	++++
	lgG2b	+++	+++
	lgG3	++	+++
	lgM	+/-	-
Rat	lgG1	-	+
	lgG2a	-	++++
	lgG2b	-	++
	lgG3	+	++
Avian egg yolk	IgY	-	-
Cow		++	++++
Dog		++	+
Goat		-	++
Guinea Pig	lgG1	++++	++
	lgG2	++++	++
Hamster		+	++
Horse		++	++++
Koala		-	+
Flame		-	+
Monkey (rhesus)		++++	++++
Pig		+++	+++
Rabbit		++++	+++
Sheep		+/-	++

Table 1 affinity data for Protein A and Protein G for different sources and subtypes of IgG. ++++ = Strong Binding; ++~+++ = Medium Binding; + = Weak Binding; +/- = Weak or No Binding; - = No Binding.

Characteristics	Description
Product content	10 mg/ml magnetic beads in specific protective buffer
Beads size	~200 nm
Magnetization	Superparamagnetic
Coupled protein	Recombinant Protein A/G
M.W. of protein	~25 kDa (Protein A/G)
Antibody concentration	≥0.6 mg Protein A/G per ml beads
Binding capacity	≥ 0.7 mg human IgG per ml beads
Specificity	Antibodies from many different species, including mouse, human, rabbit, cow, goat and sheep
Elution method	Elution with acid, competing peptide or SDS-PAGE loading buffer
Application	IP, Co-IP, Protein purification

Table 2 The main related indicators of Protein A/G beads.

Materials/composition

Component	K1305-1ml	K1305-5ml
Protein A/G beads	1 ml	5 ml

Store conditions

Store at 4 °C for two years.

Experimental operation

1. Sample preparation.

- **Adherent cells**: Discard the culture medium and wash it with PBS, normal saline, or serum-free culture medium (if the protein in the serum is not disturbed, it can be left unwashed). Add 100-200 μL of configured Cocktail-containing lysate per 0.5-1 million cells (equivalent to one well of a 6-well plate). Blowing with a gun for several times, the lysate and the cells are in full contact (under normal circumstances, the cells will be lysed after the lysate touches the animal cells for 1-2 s). Plant cells should be lysed on ice for 2-10 min. After full lysis, centrifuge 10,000-14,000g for 3-5 min, take the supernatant, and then perform subsequent operations such as PAGE, WB, IP, Co-IP, and ELISA.

- **Floating** cells: Collect cells by centrifugation, gently vortexing or flicking the bottom of the tube to disperse the cells as much as possible. Add 100-200 μL of configured Cocktail-containing lysate per 0.5-1 million cells and flick the bottom of the tube to fully lyse the cells. There should be no significant cell pellet after adequate lysis. If the cell volume is large, it must be aliquoted into 50-1 million cells/tube and then lysed. Large clumps of cells are more difficult to lyse thoroughlys, while a small number of cells are relatively easy to lyse fully because the lysate is easy to fully contact with cells. After full lysis, centrifuge 10,000-14,000g for 3-5 min, take supernatant, and then proceed to PAGE, WB, IP, Operations such as Co-IP and ELISA.
- **Bacteria or yeast**: take 1 mL of bacterial or yeast solution, centrifuge to remove the supernatant (or wash once with PBS to remove the liquid thoroughly), gently vortex or flick the bottom of the tube to disperse the bacteria or yeast as much as possible. Add 100-2 00 μL of lysate, gently vortex or flick the bottom of the tube to mix and lyse on ice for 2-10 min (if you want better lysis, bacteria and yeast can use lysozyme and wall-breaking enzyme, respectively (Lyticase) digestion followed by lysis using this lysate). After full lysis, centrifuge 10,000-14,000g for 3-5 min, take supernatant, and then proceed to PAGE, WB, IP, Operations such as Co-IP and ELISA.
- **Tissue sample: I.** Cut the tissue into fine pieces; **II.** Add lysate at the ratio of 100-200 μL of lysate per 20 mg of tissue (if the lysis is insufficient, increase the amount of lysate appropriately, if a high concentration protein sample is required, reduce the amount of lysate appropriately); **III.** Homogenize with a glass homogenizer until fully lysed. Or the tissue sample is frozen and ground with liquid nitrogen, and the lysate is added for lysis after the grinding is sufficient; **IV.** After full lysis, centrifuge 10,000-14,000 g for 3-5 min, take the supernatant, and then proceed to PAGE, WB, IP, Operations such as Co-IP and ELISA.

2. Preparation of Protein A/G beads

Since Protein A/G beads are stored in a special protective solution, they need to be properly washed before adding samples.

a. Resuspend the Protein A/G beads gently with a pipette and add 1X to 10 μl or 20 μl of bead suspension per 500 μl of sample TBS to final volume of approximately 0.5 ml. Note: If the initial volume is greater than 0.2 ml, consider placing it directly on the magnetic

shelf to separate for 10 s, remove the supernatant, and then add 1X TBS to a final volume of about 0.5 ml.

b. Gently pipette the resuspended Protein A/G beads with a pipette. Place on a magnetic rack to separate for 10 s, removing the supernatant. Repeat the above steps twice.

c. Resuspend the Protein A/G beads with 1X TBS in the amount of the initial volume.

3. Antibody binding to Protein A/G beads

a. Preparation of antibodies: dilute the antibody with 1X TBS according to the dilution ratio recommended in the antibody instructions and formulate an antibody working solution; Or formulate the antibody to a final concentration of 5-50 μg/ml of the antibody working solution. Set aside on ice. Optionally: Normal IgG working solution with the same dilution ratio or final concentration using normal IgG of the same antibody species is used to remove non-specific binding or as a negative control. Normal IgG of the same species means that if the antibody used for subsequent immunoprecipitation is mouse IgG, the appropriate amount of normal mouse IgG can be diluted with 1X TBS in this step for background reduction or as a negative control.

b. Antibody adsorption: Perform magnetic separation of the Protein A/G beads prepared in step 2, aspirate the supernatant, add 500 μl of antibody working solution or normal IgG working solution, and incubate for 15 min-1 h on a room temperature flip mixer after resuspension. *Note: It is also possible to add an appropriate amount of antibody or normal IgG directly to the Protein A/G beads in step 2 for incubation.*

c. Wash: Add 500 μl of 1X TBS and gently pipette the resuspended Protein A/G beads. Place on a magnetic rack to separate for 10 s, removing the supernatant. Repeat the wash three times. Resuspend the Protein A/G beads with 1X TBS in the amount of the initial volume. *Note: During incubation and washing, if the beads are agglomerated or flaky, it will not affect the experimental results.*

4. Immunoprecipitation (IP)

a. Remove non-specific binding (optional): Protein A/G beads bound to normal IgG prepared in step 3 are magnetically separated from the samples after 1 h incubation at 4 °C for subsequent experiments. The purpose of this experimental step is to remove proteins

that produce non-specific binding to normal IgG.

b. Samples are incubated with Protein A/G beads bound to antibodies or normal IgG. Add Protein A/G beads bound to antibody or normal IgG in the ratio of 10 µl or 20 µl of bead suspension per 500 µl protein sample, place on a side shaker or rotary mixer, and incubate at room temperature for 2 h or incubate at 4 °C overnight.

Note 1: During the incubation process, if the beads are agglomerated or flaky, it will not affect the experimental results.

Note 2: An appropriate amount of antibody or normal IgG can also be incubated overnight with the sample at room temperature for 1-2 h or 4 °C, and then 10-20 µl of bead suspension can be added for 1 h at room temperature. See FAQ 2 for details.

c. Magnetic separation. After incubation, place on a magnetic rack to separate for 10 s and remove the supernatant. *Note: Part of the supernatant can be retained for the purpose of detecting the effect of immunoprecipitation.*

d. Wash. Add 500 µl of 1X TBS and gently pipette the resuspended beads. Place on a magnetic rack to separate for 10 s, removing the supernatant. Repeat the wash three times. *Note: It is also possible to determine whether the washing is complete by detecting the OD_{280} of the washing liquid obtained, if the OD_{280} is greater than 0.05, the number of washes should be appropriately increased.*

5. Elution

According to the characteristics of the protein of interest and the subsequent experimental requirements, one of the following methods can be selected for elution.

a. Acid elution method: This method is a non-denaturing method, which is relatively fast and efficient. Eluted proteins retain their original biological activity for subsequent analytical detection.

(a) Preparation of the solution: acidic eluent (0.1 M Glycine-HCl, pH 3.0), neutralizing solution (1.0 M Tris-HCl, pH 9.0, 0.15 M NaCl).

(b) For every 10-20 µl of the original bead volume, add 100 µl of acidic eluate, mix well and place on a side shaker or rotary mixer and incubate at room temperature for 5 min. Note: The incubation time should not exceed 15 min. *Note: The volume of the*

eluate can be adjusted as appropriate, and it should be noted that the subsequent neutralizing volume also needs to be adjusted accordingly.

(c) After incubation, place on a magnetic rack to separate for 10 s, transfer the supernatant to a new centrifuge tube, and immediately add 10 μl of neutralizing solution and mix appropriately.

(d) For maximum elution efficiency, repeat steps b and c and combine the same samples.

(e) Elution and neutralization of the protein of interest is placed at 4 °C and set aside, or stored at -20 °C or -80 °C for a long time.

Note 1: Acidic elution method, although highly efficient, may still be lower than competitive elution or SDS-PAGE loading buffer elution.

Note 2: Since the difference in the protein of interest may have a certain impact on the elution efficiency of the acidic elution method, if the elution efficiency requirements are relatively high, the pH of the acidic elution can be adjusted between 2.5-3.1, and the pH value or amount of the corresponding neutralizing solution should also be adjusted, such as 100 μl *of acidic elution (0.1 M Glycine-HCl, pH 2.8) and 15 μl Neutralizing solution (1 M Tris-HCl, pH 8.5).*

b. SDS-PAGE loading buffer elution method: This method is denaturation method, and the resulting protein sample is suitable for SDS-PAGE electrophoresis or Western detection.

(a) Preparation of SDS-PAGE loading buffer: Choose the appropriate SDS-PAGE protein loading buffer and add water to make 1X. Usually SDS-PAGE protein loading buffer contains reducing agents such as DTT, and the elution of the resulting protein sample will contain light and heavy chains of antibodies.

(b) For every 10-20 μl of the original bead volume, add 100 μl of 1X SDS-PAGE loading buffer and heat at 95 °C for 5 min. Note: The volume of the eluate can be adjusted appropriately as appropriate.

(c) Place on a magnetic rack to separate for 10 s, take the supernatant for SDS-PAGE electrophoresis or Western detection.

c. Peptide competition elution method: If the protein of interest is a tagged protein and immunoprecipitation is performed using the corresponding tagged antibody, the

corresponding peptide can be used for competitive elution. This method is a non-denaturing method, the elution efficiency is high, and the eluted protein maintains the original biological activity, which is convenient for subsequent analysis and detection. The following is an example of Flag-tagged protein:

(a) Preparation of 3X Flag Peptide Eluent: Dissolve an appropriate amount of 3X Flag Peptide (A6001) in 1X TBS to give a final concentration of 150 μg/ml, or dilute 5 mg/ml of 3X Flag Peptide Solution (A6001) to 150 μg/ml.

(b) For every 10-20 μl of the original bead volume, add 100 μl of 3X Flag peptide eluate (150 μg/ml), mix well and place on a side shaker or rotary mixer, incubate at room temperature for 30-60 min, or 4 °C Incubate for 1-2 h. To improve elution efficiency, incubation time can be extended or elution repeated.

(c) After incubation, place on a magnetic rack to separate for 10 s and transfer the supernatant to a new centrifuge tube. The supernatant is the eluted Flag-tagged protein.

(d) The eluted Flag-tagged protein is placed at 4 °C and set aside or stored at -20 °C or -80 °C for a long time.

6. Co-Immunoprecipitation (Co-IP)

Refer to the method of immunoprecipitation, but co-immunoprecipitation (Co-IP) is generally appropriate to use fresh protein samples that have not been frozen, to ensure that the protein complex is not destroyed by freeze-thaw. Although cryopreserved protein samples can be used for ordinary immunoprecipitation, it is generally preferable to use fresh protein samples.

7. Purification of antibodies

a. Refer to step 3, add Protein A/G beads to the antibody sample to be purified, mix well and incubate on a side shaker or rotary mixer at room temperature for 1 h. Aspirate the supernatant and then wash 3 times with 1X TBS.

b. Referring to step 5a, elution is performed with an appropriate amount of acidic eluent and neutralized with neutralizing solution.

8. Frequently asked questions

I. How to improve the binding efficiency of antibody and magnetic beads?

The binding efficiency of the bead to the antibody is related to the species origin and subtype of the antibody, such as the affinity of the subtype of the antibody with Protein A, G or A/G is low, and the affinity can be improved by increasing the incubation time of the antibody and the bead, increasing the pH value of TBS (8-9) or reducing the ionic strength (25-100mM NaCl).

II. How can I improve the specificity of magnetic beads in immunoprecipitation or co-immunoprecipitation reactions?

- Referring to the remarks in step 4b, the appropriate amount of antibody can be incubated with the sample to form an antibody-antigen complex, and then the complex is captured with Protein A, G or A/G magnetic beads, which can improve the binding efficiency of the antibody to the antigen and reduce the time of contact between the bead and the sample, thereby improving the specificity of the precipitated product. This method is also recommended for protein/nucleic acid coprecipitation or chromatin coprecipitation.
- Referring to Step 4a, pre-incubation with protein samples using magnetic beads bound to normal IgG can reduce non-specific binding of the antibody. Similarly, it is also possible to pre-incubate with normal IgG in the protein sample, then add antibodies for incubation, and then magnetic beads for immunoprecipitation of antibodies.
- By setting up normal IgG as a control for antibodies, the specificity of immunoprecipitation or co-immunoprecipitation products can be determined.

III. How can I avoid the accumulation of beads during storage or use?

Magnetic beads should usually be kept at 2-8 °C and irreversible aggregation due to contamination should be avoided when used, or aggregation due to drying. It is normal for beads to accumulate in low-pH elution buffer and does not affect the normal use of magnetic beads. Adding a nonionic detergent with a final concentration of 0.1% (v/v) to TBS and elution buffer, such as Triton X-100, Tween-20, or NP-40, effectively prevents bead aggregation. The beads that have undergone a low-pH elution operation can be washed to neutral with TBS, and then the beads are resuspended with a TBS containing

0.1% (v/v) Tween-20 oscillation, and treated with ultrasonic water baths for 2 min, the beads can be restored to a uniform state, and the above treatment does not affect the antibody binding efficiency of the beads.

IV. How to solve the phenomenon that magnetic beads are easy to adhere to the surface of consumables such as centrifuge tubes?

It is recommended to use consumables with low adsorption rate for bead operation. In addition, adding 0.1 % (v/v) of nonionic detergents (such as Triton X-100, Tween-20, or NP-40) to the buffer can effectively reduce the adhesion of magnetic beads on the surface of consumables.

V. Do magnetic beads clump during use?

If the beads appear to be lumped when used, it is easy to cause uneven distribution. This problem occurs because the beads are placed in the magnetic field for too long so that the beads are firmly bound together. It can be redispersed by ultrasonic water bath treatment for 2 min, but it should be noted that ultrasonic treatment will also make the antibody captured by the beads in the sample solution fall off, so the beads should not be used to deal with the agglomerating of the beads before elution after dosing.

VI. Other common problems, causes and solutions:

Problem	Possible Causes	Solution
Very few or no target protein exists in the eluate.	Protein is not completely eluted.	Change elution methods.
	No target protein expressed.	Make sure the protein of interest contains the HA-tag by Western blot or dot blot analyses.
	Very low protein expression level.	1. Use larger volume of cell lysate.
		2. Optimize expression conditions to raise the protein expression level.
	Washes are too stringent.	Reduce the time and number of washes.
	Incubation times are inadequate.	Increase the incubation time.
	Interfering substance is present in sample.	Lysates containing high concentration of DTT, 2-mercaptoethanol, or other reducing agents may destroy antibody function, and must be avoided.
	Detection system is inadequate. (WB)	1. Check primary and secondary antibodies using proper controls to confirm binding and reactivity.
		2. Verify that the transfer was adequate by using prestained protein marker or staining the membrane with Ponceau S.

		3. Use fresh detection substrate or try a different detection system.
Background is too high.	Proteins bind nonspecifically to the antibody, insufficient washing on magnetic beads, or the microcentrifuge tubes.	1. Pre-clear lysate with Normal IgG to remove nonspecific binding proteins.
		2. After suspending beads for the final wash, transfer entire sample to a clean microcentrifuge tube before separation.
	Washes are insufficient.	1. Increase the number of washes.
		2. Prolong duration of the washes, incubating each wash for at least 15 minutes.
		3. Increase the salt and/or detergent concentrations in the wash solutions.
		4.Centrifuge at lower speed to avoid nonspecific trapping of denatured proteins.
Multiple protein bands found in the eluate.	The protein is not stable at room temperature.	Purify the target protein at lower temperature, such as 4 ℃.
	Protein degradation due to proteases activity during purification process.	Add protease inhibitors to cell lysate.
	Non-specific binding.	1. Prepare cell lysate again.
		2. Add additional wash steps.

Table 3 Other common problems, causes and solutions.

Notes

1. Before use, it is necessary to reverse the magnetic beads several times, the operation must be gentle, and it is not appropriate to vortex violently to avoid protein denaturation.
2. Maintain a pH of 6-8 and avoid high-speed centrifugation, drying or cryopreservation.
3. Do not place the beads in the magnetic field for a long time, otherwise they may cause the beads to accumulate.
4. After testing, repeated freeze-thaw more than 3 times does not affect the use of the effect.
5. Purification is done as soon as possible after protein sample collection and should always be placed at 4 °C or ice bath to slow protein degradation or denaturation.
6. In order to effectively inhibit protein degradation, an appropriate amount of protease inhibitor mixture can be added to the protein sample, such as protease inhibitor Cocktail-K1007, phosphatase inhibitor Cocktail-K1015, deacetylase inhibitor - K1017 and the like.
7. When the acid solution elutes, the magnetic beads may accumulate, which is a normal

phenomenon and does not affect the normal use of the magnetic beads. 0.1% nonionic detergent (such as Triton X-100, Tween-20, or NP-40) effectively prevents bead aggregation and does not affect the antibody binding efficiency of the beads. High concentrations of DTT, mercaptoethanol, guanidine hydrochloride, etc. may have a certain effect on the binding of this product to the tagged protein.

8. This product is intended for scientific use only.

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