Protein A/G Magnetic Co-IP/IP Kit

Introduction

Immunoprecipitation (IP) or Co-Immunoprecipitation (Co-IP).) is a common technique for studying proteins or protein-protein interactions (PPIs) by using specific antibodies and mediators that bind to antibodies (e.g., Protein A/G magnetic beads, etc.), or directly using a media conjugated to a specific antibody (such as agarose gel or magnetic beads) to isolate the protein of interest from the complex sample and can be used later SDS-PAGE or mass spectrometry analysis, etc.

This product is a classic magnetic bead method IP/Co-IP kit containing high-quality Protein A/G magnetic beads and optimized and validated immunoprecipitation necessary reagents, making IP or Co-IP experiments simpler, more convenient, and more convenient Highly efficient. Protein A/G can be specifically bound to the Fc terminal of the user's specific antibody and form Protein A/G magnetic beads after incubation for a certain period of time The antibody mixture (beads-Ab complex) is then added to the sample, which can be specifically recognized by the Fab terminal of the antibody to form Protein A/G magnetic beads - Antibody-antigen immune complex (Beads-Ab-Ag complex). Immune complexes are washed to remove unbound proteins, and bound immune complexes are then eluted from magnetic beads using methods such as acidic eluate or SDS-PAGE loading buffer for subsequent detection.

Protein A is a cell wall surface protein found in Staphylococcus aureus with a molecular weight of 42 kDa and is specifically associated with mammalian immunoglobulins (Immunoglobulin, Ig) Fc region will also bind to the Fab region of the human VH3 family. Protein G is a type C or G streptococcal bacteria The expressed immunoglobulin-binding protein specifically binds to the Mammalian immunoglobulin (Ig) FC region. This product is a modified recombinant Protein A (25 kDa) and Protein G (25 kDa), covalent with nanoscale amino magnetic beads Conjugation binding and retaining only the amino acid sequence associated with Fc terminal binding such as IgG removes sequences that may lead to non-specific binding outside the binding site, thereby effectively reducing non-specific binding.

Protein A beads specifically bind to corresponding antibodies, such as human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b and rabbit IgG, etc., and each Protein A molecule can bind 5 IgG molecules The antibodies that Protein G beads can bind to are human IgG1, IgG2, IgG3, IgG4, and mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c, as well as rabbit, goat polyclonal antibodies, etc., and each Protein G molecule can bind 3 IgG molecules. Protein A/G beads are mainly used for immunoprecipitation (IP) and co-precipitation (Co-IP). or Chromatin Immunoprecipitation (Ch-IP), and 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 table below (Table 1).

Protein A/G beads is a 1:1 ratio configuration of Protein A beads and Protein G beads, which has a variety of significant advantages. First, high content and binding specificity of binding antibodies can be achieved. Compared with traditional Protein A/G agarose gels, this product has a smaller pore size, is less prone to non-specific adsorption, and has a high binding amount. 1 mL of magnetic bead suspension contains approximately 10 mg of magnetic beads and not less than 0.6 mg of recombinant Protein A/G, which can bind no less than 0.7 mg Human IgG, and the specific maximum binding amount is related to the type of antibody and the target protein. For experiments, efficient immunoprecipitation is typically performed using 10-20 μL of Protein A/G beads suspension for 500 μL samples. Second, ultra-fast binding of antibodies or antibody complexes can be achieved. Protein A/G beads (~200 nm) facilitate rapid and efficient binding of magnetic beads to antibodies or antibody complexes due to their large specific surface area. Usually, the adsorption process of antibodies or their complexes can be completed within 10 minutes, and the immunoprecipitation of the target protein can be completed within 30 minutes. Shortening the operation time can effectively avoid the degradation or denaturation of the target protein during long-term operation, and fully ensure the activity of the target protein. Due to the magnetic separation, IP and Co-IP can be performed each time compared to agarose gels by 40%. Finally, a variety of methods can be used to elute. Depending on factors such as the structure, biological function, and design requirements of the subsequent application of the protein of interest, a variety of eluents such as acidic solutions, SDS-PAGE loading buffers, or competitive peptides can be used for elution purposes. (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.

Components and storage

Components	K1309-10 T	K1309-50 T
Cell Lysis Buffer	5 mL	25 mL
Protease Inhibitor Cocktail (EDTA-Free,100X in DMSO)	50 μL	250 μL
10X TBS	5 mL	30 mL
Neutralization Buffer	100 μL	500 μL
Acid Elution Buffer	1 mL	5 mL
Protein A/G beads	200 μL	1 mL
5X Protein Loading Buffer (Reducing)	200 μL	1 mL
Store Protease Inhibitor Cocktail (EDTA-Free,100X in DMSO) and 5X Protein Loading Buffer (Reducing) at -20°C and the rest of components at 4°C for 12 months.		

Experimental manipulation

1. Prepare before the experiment.

- **Configure the Cocktail-containing lysate**: Mix the Cell Lysis Buffer with the Protease Inhibitor Cocktail at 100:1 (v/v) at room temperature Configuration, on ice or 4°C place.
- **Preparation of** 1X TBS: 10X TBS is diluted to 1X with ultrapure water, which is 1X TBS. For example, 1 mL of 10X TBS is mixed with 9 mL of ultrapure water, and when mixed, it is 1X TBS.
- Preparation of 1X Protein Loading Buffer (Reducing): Take an appropriate amount of 5X Protein Loading Buffer (Reducing) and dilute it 5 times with water to make 1X Protein Loading Buffer (Reducing). For example, 0.2 mL 5X Protein Loading Buffer (Reducing) is added to 0.8 mL of ultrapure water, and when mixed, it is 1X Protein Loading Buffer (Reducing).
- **Bring your own antibodies.**

2. 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.

3. 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 Protein A/G beads by gently pipetting and suspend the turbidity solution at 10 μL or 20 μL of magnetic beads per 500 μL of sample Add an appropriate amount of Protein A/G beads to 1 clean centrifuge tube and add 1X TBS to a final volume of approximately 0.5 mL. Note: If the initial volume is greater than 0.2 mL, consider placing directly on a magnetic rack for 10 s, removing the supernatant, and then adding 1X TBS to a final volume of approximately 0.5 mL.

b. Resuspend Protein A/G beads by gently pipetting. Place on a magnetic rack to separate for 10 s and remove the supernatant. Repeat the above steps twice.

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

4. Binding of antibodies to Protein A/G beads.

a. **Preparation of antibody: Dilute the antibody with** 1X TBS according to the recommended dilution ratio in the instructions for use of the antibody to prepare the antibody working solution; Or formulate antibodies into antibody working solutions at a final concentration of 5-50 μg/mL. Keep on ice for later use. Optional: Normal IgG working solution with the same dilution ratio or final

concentration is prepared using normal IgG of the same antibody species for removal of non-specific binding or as a negative control. The so-called normal IgG of the same species means, for example, if the antibody used in subsequent immunoprecipitation is mouse IgG, the appropriate amount of normal mouse IgG can be diluted with 1X TBS in this step to lower the background or as a negative control.

b. **Antibody adsorption: Magnetic separation of the** Protein A/G beads prepared in step 3, aspirate the supernatant, and add 500 μL of antibody working solution or normal IgG working solution After resuspending incubate on a room temperature flip mixer for 15 min-1 h. *Note: It is also possible* to *incubate directly with an appropriate amount of antibody or normal IgG* to the *Protein A/G beads* in Step *3.*

c. **Wash**: Add 500 μL of 1X TBS and resuspend Protein A/G beads by gently pipetting. Place on a magnetic rack to separate for 10 s and remove the supernatant. Repeat the wash three times. Resuspend Protein A/G beads with 1X TBS in the amount of initial volume. *Note: During incubation and washing, it is normal for magnetic beads to clump or appear flaky, which will not affect the experimental results.*

5. Immunoprecipitation (IP).

a. **Remove non-specific binding** (optional): Incubate the sample with Protein A/G beads in combination with normal IgG prepared in step 4 with the sample at 4°C After 1 h, magnetic separation and supernatant samples are used for subsequent experiments. The purpose of this experimental step is to remove proteins that bind nonspecifically to normal IgG-yielding.

b. **Samples are incubated** with **Protein A/G beads** bound to antibodies or normal **IgG**: Add 10 μL or 20 per 500 μL of protein sample The ratio of μL of magnetic bead suspension is added to Protein A/G beads bound to antibodies or normal IgG, placed on a side-swing shaker or rotary mixer, and incubated at room temperature for 2 h or 4°C Incubate overnight.

Note 1: During the incubation process, if the *magnetic beads clump or appear in a flake form, it is normal to do not affect the experimental results.*

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

c. **Magnetic separation**: After incubation, place on a magnetic rack to separate for 10 s to remove the supernatant. *Note: Partial supernatant can be retained for testing 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 and remove the supernatant. Repeat the wash three times. *Note: It is also possible to determine* whether the washing is complete by detecting the OD280 of the washing liquid obtained by *washing, if* the *OD280 is greater than 0.05, the number of washings should be appropriately increased.*

6. Co-immunoprecipitation (Co-IP).

Refer to the method of immunoprecipitation, but co-immunoprecipitation (Co-IP) is usually advisable to use fresh protein samples that have not been cryopreserved to ensure that protein complexes are not destroyed by freeze-thaw. Fresh protein samples are usually preferred, although cryopreserved protein samples can be used for normal immunoprecipitation.

7. Elution:

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

a. **Acid elution method**: This method is non-denaturing, which is relatively fast and efficient. The eluted protein retains its original biological activity for subsequent analysis.

(a) Add 100 μL of Acid Elution Buffer per 10-20 μL volume of raw beads, mix well, place on a side-swing shaker or rotary mixer, and incubate for 5 minutes at room temperature. *Note 1: The incubation time should not exceed 15 min. Note 2: The volume of the eluate can be adjusted appropriately as appropriate, and it should be noted that the subsequent volume of the neutralization solution also needs to be adjusted accordingly.*

(b) 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 Neutralization Buffer to mix appropriately.

(c) For maximum elution efficiency, repeat steps a and b and combine identical samples.

(d) The protein of interest that elutes and neutralizes is placed at 4°C for use or stored at -20°C or -80°C for long periods of time.

Note 1: Acid elution methods, although highly efficient, may still be inferior to competitive elution or SDS-PAGE loading buffer elution methods.

Note 2: Since the difference of the target protein may have a certain impact on the elution efficiency of the acid elution method, if the requirements for the elution efficiency are relatively high, the *pH* of the acid eluate can be adjusted *between 2.5-3.1*, and the *pH* value or amount *of the corresponding neutralizing solution should also be adjusted, for example 100 μL of acidic eluate* (*0.1 M Glycine-HCl, pH 2.8) and 15 μL neutralizer (1M Tris-HCl, pH8.5*）.

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

(a) Add 100 μL of 1X Protein Loading Buffer (Reducing) per 10-20 μL volume of raw beads and heat at 95°C 5 min. Note: The volume of the eluate can be adjusted appropriately as appropriate.

(b) Place on a magnetic rack for separation for 10 s and take the supernatant for SDS-PAGE electrophoresis or Western detection.

c. **Competitive elution of peptides**: If the protein of interest is a tag protein and immunoprecipitated using the appropriate tag antibody, the corresponding peptide can be used for competitive elution.

This method is non-denaturing, with high elution efficiency, and the eluted protein retains the original biological activity, which is convenient for subsequent analysis and detection. The following is an example of the Flag tag protein:

(a) Preparation of 3X Flag peptide eluate: dissolve an appropriate amount of 3X Flag peptide (A6001) in 1X TBS to make its final concentration of 150 μg/mL , or dilute 5 mg/mL of 3X Flag Peptide Solution (A6001) to 150 μg/mL.

(b) Add 100 μL of 3X Flag peptide eluate (150 μg/mL) per 10-20 μL volume of raw beads. After mixing, it was placed on a side swing shaker or rotary mixer and incubated at room temperature with shaking for 30-60 min, or 4°C for 1-2 h. To improve elution efficiency, extend the incubation time or repeat the elution time.

(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 tag protein.

(d) Eluted Flag-tagged proteins are stored at 4°C for use, or at -20°C or -80°C for long periods of time.

8. Frequently asked questions:

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

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

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

- Referring to the note in step 5b, an appropriate amount of antibody can be incubated with the sample to form an antibody-antigen complex, and then the complex can be captured with Protein A, G, or A/G magnetic beads. This improves the binding efficiency of the antibody to the antigen and reduces the time the beads are in contact with the sample, thereby improving the specificity of the precipitated product. This method is also recommended for co-protein/nucleic acid precipitation or co-chromatin immunoprecipitation.
- Referring to step 5a, pre-incubation with protein samples using magnetic beads bound to normal IgG can reduce non-specific binding of antibodies. Similarly, normal IgG pre-incubation can be added to the protein sample, followed by antibody incubation, followed by magnetic beads for antibody immunoprecipitation.
- Setting normal IgG as a control for antibodies can determine the specificity of immunoprecipitation or co-precipitation products.

III. How can I avoid possible aggregation of magnetic beads during storage or use?

Magnetic beads should generally be stored at 2-8°C and should be used to avoid irreversible

aggregation due to contamination, or aggregation due to drying. It is normal for magnetic beads to aggregate in elution buffers at low pH and do not interfere with the normal use of magnetic beads. Add 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, which can effectively prevent magnetic bead aggregation. Beads that have undergone a low pH elution operation can be washed to neutral with TBS and then with one containing 0.1% (v/v) Tween-20 The magnetic beads can be restored to a uniform state by resuspending them by shaking TBS and treating them with an ultrasonic water bath for 2 min, and none of the above treatments affect the antibody binding efficiency of the magnetic beads.

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

Consumables with low adsorption rates are recommended for magnetic bead operation. In addition, the addition of 0.1% (v/v) nonionic detergents such as Triton X-100, Tween-20 or NP-40 to the buffer can effectively reduce the adhesion of magnetic beads to the surface of the consumable.

V. Magnetic beads clump during use?

If the magnetic beads are agglomerated when used, it is easy to lead to uneven distribution. This problem occurs because the beads are left in the magnetic field for too long, so that the beads are firmly bonded. The magnetic beads can be dispersed by 2 min treatment with an ultrasonic water bath, but it should be noted that ultrasonic treatment will also cause the antibody trapped by the magnetic beads in the sample solution to fall off, so it is not suitable to use this method to treat the agglomeration problem of magnetic 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	1. Pre-clear lysate with Normal IgG to remove nonspecific binding proteins.

	magnetic beads, or the microcentrifuge tubes.	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.

Note

1. Before use, it is necessary to reverse several times to mix the magnetic beads, the operation should be gentle, and it should not be violently vortexed and shaken to avoid protein denaturation.
2. Maintain pH 6-8 and avoid high-speed centrifugation, drying, or cryopreservation.
3. Do not place the beads in a magnetic field for a long time, as this may cause the beads to clump.
4. Complete follow-up work as soon as possible after protein sample collection and should always be placed at 4°C or in an ice bath to slow protein degradation or denaturation.
5. Magnetic beads may aggregate during the elution of acidic solution, which is normal and does not affect the normal use of magnetic beads. 0.1% nonionic detergents such as Triton X-100, Tween-20 or NP-40 effectively prevent magnetic bead aggregation, and it does not affect the antibody binding efficiency of the magnetic beads. High concentrations of DTT, thioethanol, guanidine hydrochloride, etc. may have a certain effect on the binding of this product to tag proteins.
6. This product is for scientific use only.

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