Immunoprecipitation Kit (Protein G Agarose Gel)

Product Description:

Immunoprecipitation (IP) or co-immunoprecipitation (Co-IP) is a common technique used to study proteins or protein-protein interactions (PPIs) by using specific antibodies and antibody-binding mediators (e.g., Protein A/ G agarose gels, etc.), or directly use a medium conjugated to a specific antibody (such as an agarose gel or magnetic beads) to separate the protein of interest from complex samples, which can then be used for Western blot detection or mass spectrometry analysis.

Protein G is an immunoglobulin-binding protein expressed by Streptococcal bacteria type C or G, which specifically binds to mammalian immunoglobulin (Ig). Fc zone binding. Protein G can bind to human IgG1, IgG2, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, IgG3, rat IgG1, IgG2a, IgG2b, IgG2c, rabbit, goat polyclonal antibodies, etc., and each Protein G molecule can bind 3 IgG molecules.

This kit contains Protein A agarose gel and other necessary reagents for immunoprecipitation, and with the specific antibodies provided by the user, it makes immunoprecipitation (IP, also known as pull-down) or co-immunoprecipitation (Co-IP) experiments simpler, more convenient and efficient, and the immunoprecipitation products can be used for the detection of the target protein or its protein complexes and other experiments.

Composition and storage conditions

Components	K4622-20 T
Cell Lysis Buffer	50 mL
Protease Inhibitor Cocktail (EDTA-Free,100X in DMSO)	0.5 mL
10X TBS	10 mL
Neutralization Buffer	200 μL
Acid Elution Buffer	2 mL
Protein G Agarose Gel	400 μL
5X Protein Loading Buffer (Reducing)	600 μL
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. Preparation before the experiment

- **Configure Cocktail-containing lysate**: Configure Cell Lysis Buffer with Protease Inhibitor Cocktail at 100:1 (v/v) at room temperature, on ice, or at 4°C.
- **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.
- **Bring your own antibodies**
- **Elution component (optional):** Tag peptide (e.g., A6001 3X FLAG Peptide).

2. Sample preparation

- **Adherent cells**: Discard the culture medium and wash it with PBS, normal saline, or serum-free culture solution (if there is no interference with the proteins in the serum, it can be left unwashed). Add 100-200 μL of prepared Cocktail-containing lysate per 50–1,000,000 cells (equivalent to one well of a 6-well plate). Pipetting with a gun several times to make the lysate fully contact with the cells (in general, 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 at 10,000-14,000 g for 3-5 minutes, remove the supernatant, and perform subsequent PAGE, WB, IP, Co-IP, and ELISA. *Note: After lysis, a small amount of insoluble substances, mainly genomic DNA, etc., will appear, and precipitates will be produced after centrifugation.*
- **Suspend cells**: Collect cells after centrifugation (250-1000 ×g, 5 min) at room temperature (if necessary, you can 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. Add 100-200 μL of Cocktail-containing lysate per 50-1,000,000 cells Gently flick the bottom of the tube to fully lyse the cells. There should be no significant cell pellet after adequate lysis. If the number of cells is large, it is necessary to aliquot into 50-1 million cells/tube before lysing. Large clumps of cells are more difficult to fully lyse, while a small number of cells are relatively easy to fully lyse due to the easy contact between the lysate and the cells. After full lysis, centrifuge at 10,000-14,000 g for 3-5 minutes, remove the supernatant, and perform subsequent PAGE, WB, IP, Co-IP, and ELISA. *Note: After lysis, a small amount of insoluble substances, mainly genomic DNA, etc., will appear, and precipitates will be produced after centrifugation.*
- **Bacteria or yeast**: Take 1 mL of bacterial or yeast solution, centrifuge to remove the supernatant (or you can wash once with PBS to remove the liquid thoroughly), and gently vortex or flick the bottom of the tube to disperse the bacteria or yeast as much as possible. Add 100–200 μL of lysate, mix by gently vortexing or bouncing the bottom of the tube, and lyse on ice for 2–10 minutes (for better lysis, bacteria and yeast can be digested with lysozyme and Lyticase, respectively, before

lysing with this lysate). After full lysis, centrifuge at 10,000-14,000 g for 3-5 minutes, remove the supernatant, and perform subsequent PAGE, WB, IP, Co-IP, and ELISA. *Note: After lysis, a small amount of insoluble substances, mainly genomic DNA, etc., will appear, and precipitates will be produced after centrifugation.*

- **Tissue sample**: I. Tissue is cut into small pieces, or if the tissue sample itself is very small, no shearing can be performed; II. Add lysate at the ratio of 100-200 µL of lysate per 10-20 mg of tissue (if lysis is insufficient, the amount of lysate can be increased appropriately, and if high-concentration protein samples are required, the amount of lysate can be appropriately reduced); III. Homogenize with a glass homogenizer until fully lysed. Or the tissue sample can be frozen and ground with liquid nitrogen, and the lysate will be added for lysis after the grinding is sufficient; After full lysis, centrifuge at 10,000-14,000 g for 3-5 min, take the supernatant, and perform subsequent PAGE, WB, IP, Co-IP, and ELISA. *Note: After lysis, a small amount of insoluble substances, mainly genomic DNA, etc., will appear, and precipitates will be produced after centrifugation.*

➢ **Note:** a. All sample lysis steps should be performed in an ice bath or at 4°C to minimize the possibility of protein degradation;Subsequent immunoprecipitation or co-immunoprecipitation should be performed immediately after sample lysis, and if subsequent experiments cannot be performed immediately, cryopreservation at -20°C or -80°C can be performed, but freeze-thaw may affect protein-protein interactions;After the sample is ready, pay attention to take a certain amount as Input or Total for subsequent Western and other detections.

The following experiment uses 100 µL of sample (pre-made Cocktail-containing lysate) as an example - steps 3, 4, 5, and 6.

3. Preparation of Protein G Agarose Gel

Since Protein G Agarose Gel is stored in a protective solution containing 50% glycerol, it needs to be properly washed before adding to the sample.

3.1 Resuspend Protein G Agarose Gel by gently pipetting with a pipette, add 20 µL to 1 clean centrifuge tube, and add 1X TBS to a final volume of approximately 0.5 mL.

3.2 Resuspend with a pipette gently after centrifugation at 6,000 ×g for 30 s at 4°C, carefully removing the supernatant but not aspirating to the gel. Repeat steps (3.1 and 3.2) twice.

3.3 Resuspend Protein G Agarose Gel with 1X TBS as per the initial volume.

4. Binding of antibodies to Protein G Agarose Gel

4.1 **Preparation of antibodies**: Prepare antibody working solution by diluting the antibody with 1X TBS at the dilution ratio recommended in the antibody instructions for use, or prepare the antibody into an antibody working solution at a final concentration of 5-50 µg/mL. Set aside on ice. Optional: Prepare a separate aliquot of antibody, i.e., prepare the same dilution or final concentration of normal IgG using the same normal IgG of the antibody species for non-specific

binding or as a negative control. The so-called normal IgG of the same species means, for example, that the antibody used in subsequent immunoprecipitation is mouse IgG, and the appropriate amount of normal mouse IgG can be diluted with 1X TBS in this step to reduce background or as a negative control.

4.2 **Antibody adsorption**: Centrifuge the Protein G Agarose Gel prepared in step 3 (6000×g, 4°C, 30 s), aspirate the supernatant, do not aspirate to the gel, and add 100 μL of antibody working solution or normal IgG working solution, resuspended, incubated on a room temperature inversion mixer for 15 min-1 h. *Note: You can also add an appropriate amount of antibody or normal IgG directly to the Protein G Agarose Gel in step 3 for incubation.*

4.3 **Wash**: Add 500 μL of 1X TBS and resuspend Protein G Agarose Gel. Immerse on ice and place on a shaker for 5 min, then centrifuge at 6,000 ×g at 4°C for 30 s, carefully removing the supernatant without aspirating to the gel. Repeat the wash three times. Resuspend Protein G Agarose Gel with 1X TBS as per the initial volume.

5. Immunoprecipitation (IP)

5.1 **Removal of non-specific binding** (optional): The Protein G Agarose Gel prepared in step 4 with normal IgG (e.g., normal mouse IgG) was incubated at 4°C for 1 h, centrifuged, and the supernatant samples were used for subsequent experiments (5.2). The purpose of this protocol is to remove proteins from the sample that bind non-specifically to normal IgG.

5.2 **Incubate samples with Protein G Agarose Gel bound to antibody or normal IgG:** Add 20 μL of Protein A Agarose bound to antibody or normal IgG at 100 μL of protein sample (5.1 supernatant or untreated 5.1). Gel, placed on a side-swinging shaker or rotary mixer, and incubated overnight at room temperature for 2 h or 4°C.

[Note 1]: During the incubation process, if the agarose gel clumps or is flaky, it is normal and will not affect the experimental results.

[Note 2]: You can also incubate an appropriate amount of antibody or normal IgG with the sample at room temperature for 1-2 h or 4°C overnight, and then add 10-20 μL of gel suspension and incubate at room temperature for 1 h. See FAQ 7-II for details.

5.3 **Centrifugation**: After incubation, centrifuge at 6,000×g at 4°C for 30 s to remove the supernatant. *Note: Part of the supernatant can be retained for testing the effect of immunoprecipitation.*

5.4 **Wash**: Add 500 μL of 1X TBS and gently pipette to resuspend the gel. Immerse on ice and place on a shaker for 5 min, then centrifuge at 6,000 ×g at 4°C for 30 s, carefully removing the supernatant without aspirating to the gel. *[Note]: You can also judge whether the washing is complete by testing the OD280 of the washing solution obtained by washing, if the OD280 is greater than 0.05, the number of washing should be increased appropriately.*

6. Elution

According to the characteristics of the target protein and the requirements of subsequent experiments, one of the following methods can be selected for elution (**choose one of the three below**).

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

(a) After washing, 100 μL of Acid Elution Buffer was added to the tube, mixed well, placed on a side swinger or rotary mixer, and incubated at room temperature for 5 minutes. *[Note 1]: The incubation time should not exceed 15 min. [Note 2]: The volume of the eluent can be adjusted appropriately as appropriate, and it should be noted that the volume of the subsequent neutralizing liquid also needs to be adjusted accordingly.*

(b) After incubation, centrifuge at 6,000×g at 4°C for 30 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, steps A and B can be repeated and the same samples combined.

(d) The eluted and neutralized protein of interest is stored at 4°C for use or at -20°C or -80°C for long-term storage.

[Note 1]: Although the acid elution method is efficient, it may still be lower than the competition elution method or the SDS-PAGE loading buffer elution method.

[Note 2]: Since the difference in the target protein may have a certain impact on the elution efficiency of the acid eluent method, if the requirements for the elution efficiency are relatively high, the pH of the acid eluent can be adjusted between 2.5-3.1, and the pH value or amount of the corresponding neutralization solution should also be adjusted to a certain extent, for example, 100 μL of acid eluent (0.1M Glycine-HCl, pH 2.8) and 15 μL neutralization solution (1 M Tris-HCl, pH 8.5).

6.2 **SDS-PAGE loading buffer elution method**: This method is a denaturation method, and the obtained protein samples are suitable for SDS-PAGE electrophoresis or Western blot detection.

(a) After washing, 5 μL of 5X Protein Loading Buffer (Reducing) was added to the tube and heated at 95°C for 5 min. *Note: The volume of the eluent can be adjusted as appropriate.*

(b) Centrifuge at 6000×g at 4°C or room temperature for 30 s, and take the supernatant for SDS-PAGE electrophoresis or Western blot detection.

6.3 **Peptide competitive elution**: If the protein of interest is a tagged protein and the corresponding tag antibody is used for immunoprecipitation, the corresponding peptide can be used for competitive elution. This method is a native method, 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 a Flag-tagged protein:

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

(b) After washing, 100 μL of 3X Flag peptide eluent (150 μg/mL) was added to the tube, mixed well, placed on a side swinger or rotary mixer, and incubated at room temperature for 30-60 minutes with shaking, or 1-2 h at 4°C. To improve elution efficiency, the incubation time can be extended or the elution can be repeated. *Note: The volume of the 3X Flag peptide eluent is generally 5 times that of the gel suspension.*

(c) After incubation, centrifuge at 6,000 ×g at 4°C for 30 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 stored at 4°C or -20°C or -80°C for long-term storage.

7. Frequently asked questions, FAQs.

I. How can I improve the binding efficiency of my antibody to an agarose gel?

The binding efficiency of the agarose gel to the antibody is related to the species source and isoform of the antibody, if the isoform of the antibody has a low affinity for Protein A, G or A/G, the affinity can be increased by increasing the incubation time of the antibody with the agarose gel, increasing the pH of TBS (8-9), or decreasing the ionic strength (25-100 mM NaCl).

II. How can I improve the specificity of an agarose gel in an immunoprecipitation or co-immunoprecipitation reaction?

- Referring to the note in step 5b, you can first incubate the appropriate amount of antibody with the sample to form an antibody-antigen complex, and then capture the complex with Protein A, G, or A/G agarose gel, which can improve the binding efficiency of the antibody to the antigen and reduce the contact time of the agarose gel with the sample, thereby improving the specificity of the precipitated product. This method is also recommended for protein/nucleic acid co-precipitation or chromatin immunoprecipitation.
- Referring to step 5a, pre-incubation with a protein sample using an agarose gel bound to normal IgG can reduce non-specific binding of antibodies. Similarly, protein samples can be pre-incubated with normal IgG, followed by antibodies, and then by agarose gels for antibody immunoprecipitation.
- The specificity of the immunoprecipitated or co-immunoprecipitated product can be determined by setting up normal IgG as a control for the antibody.

III. How can I avoid aggregation of agarose gels during storage or use?

Agarose gels should generally be stored at 2-8°C and should be used to avoid irreversible aggregation due to contamination, or aggregation due to drying. Aggregation of agarose gels in the elution buffer at low pH is normal and does not affect the normal use of agarose gels. The addition of a nonionic detergent, such as Triton X-100, Tween-20, or NP-40, to TBS and elution buffer at a final concentration of 0.1% (v/v) is effective in preventing agarose gel aggregation. Agarose gels that have undergone a low-pH elution operation can be washed to neutral with TBS, then

resuspended with 0.1% (v/v) Tween-20 with TBS shaking and treated with an ultrasonic water bath for 2 minutes to restore the agarose gels to a homogeneous state, all of which do not affect the antibody binding efficiency of the agarose gels.

IV. How to solve the phenomenon that agarose gels tend to adhere to the surface of consumables such as centrifuge tubes?

It is recommended to use consumables with low adsorption rates for agarose gel manipulation. In addition, the addition of 0.1% (v/v) of a nonionic detergent (e.g., Triton X-100, Tween-20, or NP-40) to the buffer can effectively reduce the adhesion of the agarose gel to the surface of the consumables.

V. How do I address the phenomenon of little or no target protein in the eluent?

If the protein is not completely eluting, the elution method can be changed, if there is no target protein expression, Western blot or dot blot analysis can be performed to ensure that the target protein contains the HA tag, if the protein expression level is very low, a larger volume of cell lysate or expression conditions can be optimized to increase the protein expression level, if the number of washes or time is too much, the time and number of washes can be reduced, if the incubation time is insufficient, the incubation time can be increased, if there are interferences in the sample, such as containing high concentrations of DTT, 2- Lysates from mercaptoethanol or other reducing agents may disrupt antibody function and can be avoided if the detection system is incorrect, check the primary and secondary antibodies with appropriate controls to confirm binding and reactivity, or use a prestained protein marker or Ponceau S stained membrane to verify adequate transfer, or use a fresh detection substrate or try a different detection system to resolve.

VI. How to solve the problem of too high background?

If the protein binds non-specifically to the antibody and the beads or microcentrifuge tube wash is inadequate, the final wash with normal IgG pre-clarified lysate to remove the non-specifically bound protein or suspension beads can be used, and the whole sample can be transferred to a clean microcentrifuge tube before separation. or detergent concentration, or low-speed centrifugation to avoid non-specific capture of denatured proteins.

VII. How do I fix the problem of multiple protein bands being found in my eluent?

If the protein is unstable at room temperature, the protein of interest can be purified at a lower temperature (e.g., 4°C), protease inhibitors can be added to the cell lysate if the protein is degraded due to protease activity during purification, and if non-specific binding is not required, the cell lysate can be prepared again, or an additional wash step can be added.

Precautions

1. Before use, it is necessary to mix the agarose gel by inverting several times, and the operation must be gentle, and it is not appropriate to vortex violently to avoid protein denaturation.
2. Follow-up should be done as soon as possible after the protein sample is collected and should always be left at 4°C or in an ice bath to slow down protein degradation or denaturation.
3. If centrifugation does not completely remove insoluble matter from the protein sample, the sample solution can be filtered through a 0.45 μm filter.
4. This product is for scientific use only.

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