Immunoprecipitation Kit (Protein G Magnetic Beads)

I1506975

Storage 2-8℃,-20℃. For detailed storage conditions, please refer to the Kit Contents.

Introduction

The Aladdin Immunoprecipitation Kit (Protein G Magnetic Beads) provides a classic magnetic bead -based solution for Immunoprecipitation (IP,Co-IP) experiments.

The technique utilizes a specific antibody to recognize and bind the target protein (antigen) in solution. This is followed by the use of a medium that efficiently binds antibodies such as the high-quality recombinant Protein G provided in this kit to form an insoluble antigen-antibody-bead complex. Through magnetic separation rack, the target protein can be specifically captured and purified from complex sample mixtures, such as cell lysates.

Based on this principle, the precipitated products obtained with this kit are suitable for subsequent analysis of the target protein or its interacting complex components. It serves as an ideal pre-processing tool for downstream detection techniques such as Western Blot verification and mass spectrometry analysis. The kit's core components include optimized recombinant Protein G Magnetic Beads and necessary supporting reagents, ensuring a convenient, stable, and efficient experimental process.

The Protein G Magnetic Beads in this kit can specifically bind to relevant antibodies and can be easily applied with a centrifuge for experiments such as immunoprecipitation or purification of the target protein or its protein complexes. Its features include:

(1) High specificity and high target protein binding capacity. The Protein G Magnetic Beads provided in this kit is a 10% suspension of agarose magnetic beads. Each milliliter of settled Protein G Magnetic Beads can bind over 30-40 mg of human IgG, exhibits high specificity, and has low non-specific protein binding.

(2) Reusable multiple times, cost-effective. Under normal conditions, this product can be recovered and reused 3-5 times for purification with the same antibody. Reuse is not recommended when used for co-immunoprecipitation to detect protein-protein interactions.

Kit Contents

I1506975	Component	20 T	100 T	Storage conditions	Quantity Per Test
I1506975A	IP Lysis Buffer	50 mL	250 mL	2-8°C	200μL
I1506975B	TBS(10×)	10 mL	30 mL	2-8°C	50μL
I1506975C	IP Protein G Magnetic Beads	0.4 mL	2 mL	2-8°C	20μL per 500~1000μg lysate
I1506975D	Mouse IgG	10 μL	50 μL	-20°C	On Request
I1506975E	Rabbit IgG	10 μL	50 μL	-20°C	On Request
I1506975F	IP Elution Buffer	2 mL	10 mL	2-8°C	50μL
I1506975G	IP Neutralization Buffer	0.2 mL	1 mL	2-8°C	5μL
I1506975H	SDS-PAGE Loading Buffer(5×)	0.4 mL	2 mL	-20°C	20μL

Instruction for use

1. Sample Preparation：(Note: Perform all sample lysis steps at 4°C or on ice)

(1) For Serum Samples:

If the target protein is abundant, dilute the serum sample with Lysis Buffer to a final target protein concentration of 50–150 μg/mL. Keep the diluted sample on ice for immediate use or store at -20°C for long-term preservation.

(2) For Adherent Cell Lysis and Preparation:

Aspirate the culture medium and wash the cells twice with PBS. Remove all residual liquid completely. Add 100-200 μL of Lysis Buffer with inhibitors per 0.5-1 million cells (equivalent to one well of a 6-well plate). Pipette gently to ensure thorough contact between the lysis buffer and cells. Animal cells are typically lysed within 1-2 seconds of contact with the buffer. For plant cells, lyse on ice for 2-10 minutes. After complete lysis, use a cell scraper to detach the cells and transfer the lysate to a 1.5 mL microcentrifuge tube. Centrifuge at 10,000-14,000×g for 3-5 minutes at 4°C. Collect the supernatant for protein concentration determination before proceeding to subsequent immunoprecipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, may be present after lysis and will form a pellet upon centrifugation.

(3) For Suspension Cell Lysis and Preparation:

Collect cells by centrifugation at 250-1,000×g for 5 minutes at room temperature. Wash the pellet twice with PBS and remove all residual liquid completely. Gently vortex or tap the tube to disperse the cells. Add 100-200 µL of Lysis Buffer with inhibitors per 0.5-1 million cells. Mix and incubate on ice for 5-20 minutes (mix several times during incubation). Tap the tube or pipette gently to ensure complete cell lysis; no significant cell pellet should remain after thorough lysis. If processing a large number of cells, it is recommended to aliquot them into tubes containing 0.5-1 million cells per tube before lysis. Large cell clumps are more difficult to lyse completely, whereas smaller numbers of cells allow better contact with the lysis buffer and lyse more efficiently. After complete lysis, centrifuge at 10,000-14,000×g for 3-5 minutes at 4°C. Collect the supernatant for protein concentration determination before subsequent immune-precipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, may be present after lysis and will form a pellet upon centrifugation.

(4) For Bacterial or Yeast Sample Lysis and Preparation:

For 1 mL of bacterial or yeast culture, centrifuge to pellet the cells and remove the supernatant. Wash the pellet twice with PBS and remove all residual liquid completely. Gently vortex or tap the tube to resuspend and disperse the bacterial or yeast cells. Add 100-200 µL of Lysis Buffer with inhibitors. Gently vortex or tap the tube to mix, and lyse on ice for 2-10 minutes. For improved lysis efficiency, bacteria and yeast can be pretreated with lysozyme and lyticase, respectively, before adding the Lysis Buffer with inhibitors. After complete lysis, centrifuge at 10,000-14,000×g for 3-5 minutes at 4°C. Collect the supernatant for protein concentration determination before subsequent immunoprecipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, is likely present after lysis and will form a pellet upon centrifugation.

(5) For Tissue Sample Lysis and Preparation:

Mince the tissue into small fragments. Add approximately 100-200 µL of Lysis Buffer per 20 mg of tissue. Homogenize the mixture using a glass homogenizer or other suitable homogenization device. Thorough homogenization ensures complete tissue lysis. After lysis, centrifuge at 12,000×g for 5 minutes at 4°C. Collect the supernatant for protein concentration determination before subsequent immunoprecipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, is likely present after lysis and will form a pellet upon centrifugation.

2. Immune Complex Formation:

(1) Combine 2-10 µg of affinity-purified antibody (diluted with 1×TBS) with the pre-cleared cell lysate in a microcentrifuge tube. The recommended total protein amount per IP

reaction is 500–1000 μg, as determined by a BCA protein assay kit.

(2) Dilute the cell lysate mixture with IP Lysis Buffer to a final volume of 300–600 μL.

(3) Incubate on a rotator or rocking platform at 4°C for 1 hour to overnight to allow immune complex formation.

Note: The required sample amount and incubation time are dependent on the specific antibody-antigen system and may require optimization to maximize yield. The above protocol is designed for 2-10 μg of affinity-purified antibody and can be scaled up as needed.

3. Protein G Magnetic Beads Preparation:

(1) Gently resuspend the Protein G Magnetic Beads to form a uniform suspension as much as possible. Transfer 20 μl of the suspension to a clean microcentrifuge tube and add pre-chilled 1×TBS to a final volume of approximately 0.5 mL-1 mL.

(2) Place the tube on a magnetic stand for 10 seconds to separate the beads, then carefully remove and discard the supernatant. Avoid disturbing the bead pellet. Repeat this wash step twice.

4. Capture of Immune Complexes:

(1) Binding: Add the antigen-antibody complex mixture to the prepared Protein G Magnetic Beads. Incubate on a rotator or rocking platform at 4°C for 1-4 hours.

(2) Washing: Place the tube on a magnetic stand for 10 seconds to separate the beads, then carefully remove and discard the supernatant. Avoid disturbing the bead pellet. Repeat this wash step twice.

(3) Elution: This kit provides two elution protocols. Users can select the appropriate method based on downstream application requirements. Denaturing elution is suitable for Western blot analysis. Non-denaturing elution, after neutralization, is suitable for enzymatic assays or functional studies.

a. Eaturing Elution:

This method is suitable for SDS-PAGE analysis. Add 20 μL of 1 × SDS-PAGE Loading Buffer(Dilute with 5× loading buffer) to the washed Protein G Magnetic Beads containing the antigen-antibody complexes. Mix thoroughly. Heat at 100°C for 10 minutes. After cooling, place the tube on a magnetic stand for 10 seconds to separate the beads. Collect the supernatant for SDS-PAGE analysis.

b. Non-denaturing Elution:

This method preserves the native biological activity of the eluted samples for subsequent functional analysis. Add 50 μL of Elution Buffer to the washed Protein G Magnetic Beads containing the antigen-antibody complexes. Mix and incubate on a rotator at room temperature for 5 minutes. After incubation, place the tube on a magnetic stand for 10 seconds to separate

the beads. Immediately transfer the supernatant to a new microcentrifuge tube and add 5 µL of Neutralization Buffer. Mix well to adjust the pH of the eluate to neutral for subsequent functional analysis.

Notes:

a. To maximize elution efficiency, repeat the elution step and pool the eluates.

b. Eluted and neutralized proteins/complexes can be stored at 4°C for immediate use or at -20°C/-80°C for long-term storage.

c. Although acidic elution is efficient, it may be less effective than denaturing elution.

d. Elution efficiency of the acidic method may vary depending on the target protein. If high elution efficiency is critical, optimize the pH of the elution buffer (with corresponding adjustments to the neutralization buffer). Alternatively, consider using potentially more efficient methods such as peptide competition elution or the highly efficient denaturing elution. Note that denaturing conditions may impact subsequent experiments requiring protein activity.

e. Add an additional 250 µL of PBS containing 2% FBS, mix gently, and proceed to detection using an appropriate flow cytometry instrument.

Matters needing attention

1. The IP Lysis Buffer included in this kit contains protease inhibitors. If specific experimental requirements exist, consider adding other appropriate inhibitor cocktails.
2. All sample lysis procedures should be performed on ice or at 4°C to minimize potential protein degradation. If cell lysis is insufficient, it can be supplemented with sonication after adding the lysis buffer.
3. The IP Lysis Buffer provided in this kit has been extensively tested and is suitable for sample lysis and subsequent washing in many immunoprecipitation scenarios. However, due to the complexity and specificity of immunoprecipitated protein samples, this lysis buffer may not be optimal for all IP sample types. If suboptimal performance is observed with the provided lysis or wash buffers during experimentation, optimization of lysis and wash conditions should be performed by the user.
4. The Magnetic Beads must be fully resuspended before use by inverting the vial several times to ensure a homogeneous mixture.
5. Protein samples should be purified as soon as possible after collection and must always be kept at 4°C or on ice to slow protein degradation or denaturation.
6. When using this kit, the antibody will be co-eluted with the immunoprecipitated antigen. Consequently, during reducing SDS-PAGE or Western blot analysis, at least three protein bands will be detected: the antibody heavy chain (approximately 50 kDa), light

chain (approximately 25 kDa), and the antigen. If the antibody bands obscure the immunoprecipitated antigen, Western blot detection can be performed using an antibody from a different species than that used for immunoprecipitation (for example, using rabbit-derived IgG for detection if mouse-derived IgG was used for immunoprecipitation). Alternatively, Aladdin's conformation-specific secondary antibodies (rp303272) can be purchased to directly avoid interference from antibody heavy and light chains in IP experiments.

7. The lysis buffer provided in this kit is used for both sample lysis and the subsequent washing steps.