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Immunoprecipitation (IP) Kit

rev. 4/18

(Catalog # K286-25; 25 assays; Store at 4 °C)

I. Introduction:

Immunoprecipitation (IP) is widely used in research and development, by which a protein can be selectively purified from samples. BioVision's immunoprecipitation kit provides optimized buffers for preparing cell/tissue extracts, antigen binding and washing steps. The protein A/G Sepharose beads provided in the kit has higher binding capacity with broader antibody isotype binding than traditional protein A or protein G resins. The kit can be used in variety of immunoprecipitation or Co-IP studies.

II. Application:

- Immunoprecipitation (IP) and Co-IP
- Functional study of Immunoprecipitated proteins/complexes
- SDS-PAGE or western blot analysis of Immunoprecipitated proteins/complexes

III. Sample Type:

- Tissue or cell extracts
- Biological samples

IV. Kit Contents:

Components	K286-25	Cap Code	Part Number
Non-Denaturing Lysis Buffer	40 ml	Clear NM	K286-25-1
RIPA Lysis Buffer	40 ml	Blue NM	K286-25-2
Protease Inhibitor Cocktail (lyophilized)	1 vial	Red	K286-25-3
10X Wash Buffer	20 ml	Clear WM	K286-25-4
Protein A/G Sepharose	1 ml	Orange	K286-25-5

V. User Supplied Reagents and Equipment:

- Primary antibody to the targeted protein
- Rotary mixer
- Phosphate Buffered Saline (PBS), 1M Tris/HCl pH 8.5

VI. Storage and Handling:

- Store kit at 4°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the experiment.

VII. Reagent Preparation and Storage Conditions:

- **Non-Denaturing Lysis Buffer and RIPA Lysis Buffer:** Store buffer at 4˚C once opened. Add 2 µl protease inhibitor cocktail per ml of Non-Denaturing Buffer just before use. Make fresh each time.
- **Protease Inhibitor Cocktail:** Re-suspend in 250 µl of DMSO. Aliquot and store at -20°C.
- **10X Wash Buffer:** To make 1X buffer add 1 ml of 10X buffer to 9 ml deionized water.
- **Protein A/G Sepharose:** Store at 4˚C once the kit is opened.

VIII. Immunoprecipitation (IP) Protocol:

1. Sample Preparation:

Cell Extracts: For adherent cells, remove media and wash cells with PBS. Place culture plate on ice; add cold Lysis Buffer. Keep the plate on ice for one minute. Scrape the cells and gently transfer the disrupted cell suspension into a chilled microcentrifuge tube. Mix on a rotary mixer at 4°C for 30 min. Centrifuge at 10,000 g for 10 min. at 4°C & transfer the cell extract to chilled fresh tubes. For Suspension Cells, collect cells by centrifugation. Wash cells with PBS at room temperature and collect cells again by centrifugation. Drain the PBS carefully and prepare the cell lysates as described for adherent cells.

Notes:

a) The no. of cells needed for optimal immunoprecipitation depends on the concentration of target antigen present in the sample and the affinity of the antibody to the antigen.

b) Use Non-Denaturing Lysis Buffer for maintaining protein activity, studying protein-protein interaction & for antigens that are detergent soluble and can be recognized in the native form by the antibody. This buffer can be used for IP and Co-IP. The RIPA buffer, more denaturing than the Non-Denaturing Buffer, has 0.1% SDS, 1% NP40 and 0.5% Deoxycholate and can be used for an IP and may work for a Co-IP depending on how tight the complexes are.

c) Lysis Buffer guidelines: 100-200 µl/well (24-well plate), 250-400 µl/well (6-well plate), 250-500 µl (100 x 60 mm dish) or 500-1000 µl (100 x 100 mm) dish.

d) Butt end of a pipette tip can be used to scrape cells from wells.

Tissue Extracts: Snap Freeze dissected tissue and immediately grind to a fine powder using a mortar and pestle in a liquid nitrogen bath. Transfer the ground tissue to a pre-weighed chilled tube. Weigh the powder and store at -80°C until use. Add 300 µl Lysis Buffer with protease inhibitors per 5 mg of tissue powder. Mix on a rocker at 4˚C for about an hour. Pass the lysate through a 25 gauge needle 3X. Collect the lysate and centrifuge at high speed (10,000g) at 4˚C for 5 min. to remove cell debris. Transfer the tissue extract (supernatant) to a fresh tube.

Note: Other preferred methods can be used to prepare cell/tissue extracts using the Lysis Buffers provided.

2. **Antibody Binding:** Add a predetermined amount (µg) of antibody (as recommended by the vendor or titrated by user) against the target to a known amount (µg) of sample (as standardized by user). Make up the volume to 500 µl with Lysis Buffer containing protease inhibitors. Gently mix 3-4 hrs. or overnight at 4°C on a rotary mixer.

   Notes:

   a) Incubation time depends on the affinity of the antibody to the antigen.

   b) Starting amount of sample should be in the range of 10-1000 µg protein.

   c) If desired, use the same amount of non-specific antibody as the control (use the same species as the antibody used for the IP) for the same amount (µg) of cell lysate as in the samples. For example: if you are using a rabbit anti-HDAC antibody for the IP, use Rabbit IgG as the non-specific antibody for the control.

3. **Preparation of Protein A/G Beads:** Wash the Protein-A/G beads (25-40 µl/reaction) twice with 1ml Wash Buffer centrifuging at 2000g for ~2 minutes and aspirating the supernatant in between washes. Suspend as 50% slurry in Wash Buffer. 25 µl Protein A/G Sepharose beads can bind over 500 µg IgG.

   Notes:

   a) Use wide orifice pipette tips or tips with the end cut off when pipetting beads.

   b) Beads can be used as-is or blocked using 2 volumes 5% BSA (not provided) in PBS to block possible non-specific binding if desired.

4. **Bead Capture:** After Antibody Binding (step 2), add 25-40 µl of protein-A/G beads slurry to each tube and incubate for an hour at 4°C. Collect the beads by low speed centrifugation at 4°C (2000g for 2 min). Wash beads 3X with 1ml Wash Buffer, collecting the beads by low speed centrifugation at 4°C and aspirating the supernatant in between washes. After the last wash, remove as much of the Wash Buffer as possible making sure that the beads never dries completely.

5. **Elution:**

   a) Functional Assay: the beads with the antigen-antibody (Ag-Ab) complex may be directly used for an activity assay provided the antibody does not block the active site of the protein being assayed.

   b) SDS Buffer (denaturing) elution: add 40 µl 2x SDS-PAGE loading buffer (not provided) to the beads and boil for five minutes to elute the complex. Centrifuge to collect eluent. Eluent can be stored on ice for same day analysis or frozen at -80˚C for future analysis by SDS-PAGE.

   c) Low-pH (non-denaturing) elution**:** add 40 µl low pH glycine buffer (100 mM glycine/HCl, pH 2.5-3.0, not provided), incubate for 10 min. at room temperature with frequent agitation. Centrifuge to collect the eluent & perform an additional elution as needed. Add 1/10th the volume of 1M Tris/HCl pH 8.5 (not provided) to the eluent to neutralize the low pH, store the eluent at -80°C until use. This buffer dissociates most protein-protein and antibody-antigen interactions without affecting protein structure. Some antibodies and proteins may be damaged by low pH. The affinity purified protein may be used for an activity assay.

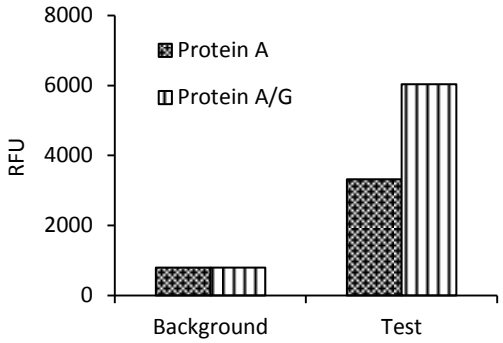

Figure. Comparison of Binding Capacity of Protein-A and Protein-A/G Beads. HDAC2 IP-Activity Assay of Ag-Ab Complex captured on Protein A or Protein A/G beads. Protein-A/G beads IP better than protein-A beads.

IX. RELATED PRODUCTS:

Protein A	Protein G
Protein L	Protein A/G
Protein A/G/L	Protein A, G and L antibody
Hi-Bind™ Protein A-Agarose	Protein A-Agarose
Protein A-Sepharose®	Protein A Magnetic Beads
Protein A-Sepharose® Column	Protein G-Sepharose® Column
Protein A/G Sepharose®	Protein G-Sepharose®
Protein A/G Magnetic Beads	Hi-Bind™ Protein G Agarose
Protein A/G-FITC	Protein G Coated plates
Protein L Magnetic Beads	Protein G Magnetic Beads
Protein L Sepharose®	Protein L-Sepharose® Column
Protein A/G/L-magnetic beads	Protein A/G/L-Sepharose®
Rabbit IgG, Rabbit Serum	Cell Lysis Buffer, 3X SDS-Page Sample Buffer
Anti-Rabbit, Mouse & Goat IgG-HRP linked Antibody	

FOR RESEARCH USE ONLY! Not to be used on humans.