ab206996
Immunoprecipitation kit

Instructions for use:

For efficient immunoprecipitation (IP) and co-immunoprecipitation (Co-IP).

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 10a, Last Updated 15 March 2024

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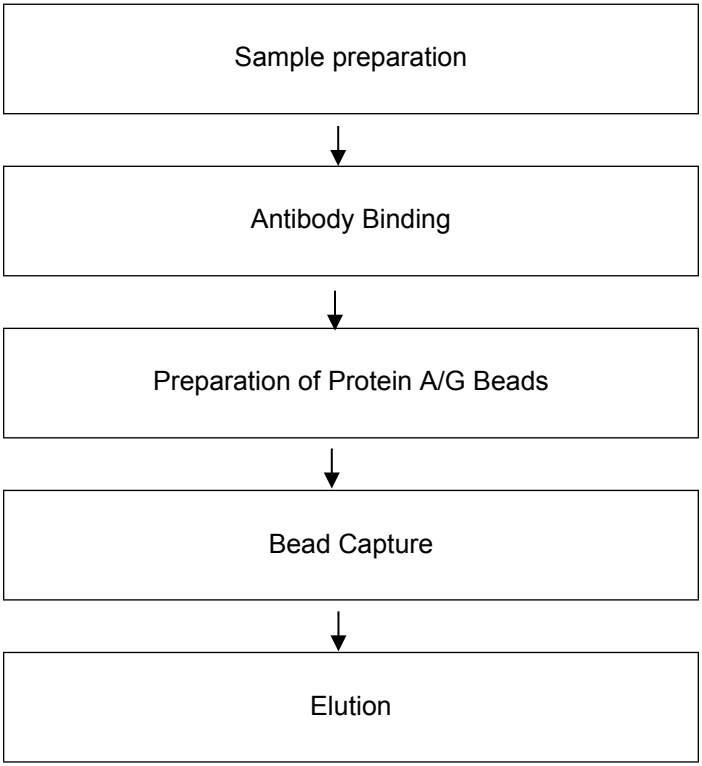
1. BACKGROUND

Abcam's Immunoprecipitation Kit (ab206996) can be used to perform immunoprecipitation (IP) and Co-IP for functional studies of immunoprecipitated proteins/complexes and SDS-PAGE or western blot analysis of immunoprecipitated proteins and complexes.

Abcam's Immunoprecipitation Kit provides optimized buffers for preparing cell/tissue extracts, antigen binding and washing steps. The Protein A/G Sepharose Beads I/Protein A/G Sepharose® beads provided in the kit have a higher binding capacity with broader antibody isotype binding than traditional Protein A or Protein G resins.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 4ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 6 and 9.

5. LIMITATIONS

- Kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Non-Denaturing Lysis Buffer/Lysis Buffer (non-denaturing)	40 mL	4°C	4°C
RIPA Lysis Buffer	40 mL	4°C	4°C
Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail (lyophilized)	1 vial	-20°C	-20°C
10X Wash Buffer X/10X Wash Buffer	20 mL	4°C	4°C
Protein A/G Sepharose Beads I/Protein A/G Sepharose®	1 mL	4°C	4°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Primary antibody to the targeted protein
- Rotary mixer
- Phosphate Buffered Saline (PBS)
- DMSO
- 1M Tris/HCl pH 8.5
- 2 X SDS-PAGE loading buffer
- 100 mM glycine/HCl, pH 2.5-3.0

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single immunoprecipitation experiment. The starting amount of tissue or cells for a single experiment will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- The number 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.
- Use Non-Denaturing Lysis Buffer/Lysis Buffer (non-denaturing) for maintaining protein activity, studying protein-protein interaction and 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. RIPA Lysis Buffer is more denaturing than the Non-Denaturing Lysis Buffer/Lysis Buffer (non-denaturing) and contains 0.1% SDS, 1% NP40 and 0.5% Deoxycholate. It can be used for IP and may work for Co-IP, depending on how tightly bound the complexes are.
- 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.
- The end of a pipette tip or a cell scraper can be used to scrape cells from wells.
- Other methods can be used to prepare cell/tissue extracts using the Lysis Buffers provided.
- Incubation time for antibody binding depends on the affinity of the antibody for the antigen.
- Starting amount of sample should be in the range of 10-1000 µg protein.

- If desired, use the same amount of non-specific antibody as a 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.
- Use wide orifice pipette tips or tips with the end cut off when pipetting beads.
- Beads can be used as provided or blocked using 2 volumes 5% BSA (not provided) in PBS to block possible non-specific binding if desired.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1. **Non-Denaturing Lysis Buffer/Lysis Buffer (non-denaturing):**

Store the buffer at 4˚C once opened. Add 2 µL Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail per mL of to the required amount of Non-Denaturing Lysis Buffer/Lysis Buffer (non-denaturing) just before use.

9.2. **RIPA Lysis Buffer:**

Store the buffer at 4˚C once opened. Add 2 µL Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail per mL to the required amount of RIPA Lysis Buffer just before use.

9.3. **Protease Inhibitor Cocktail:**

Resuspend the lyophilized Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail in 250 µL DMSO (not provided). Aliquot and store at -20°C.

9.4. **10X Wash Buffer X/10 X Wash Buffer:**

To make 1 X buffer add 1 mL of 10 X Wash Buffer X/buffer to 9 mL deionized water just before use.

9.5. **Protein A/G Sepharose Beads I/Protein A/G Sepharose®:**

Ready to use as supplied. Store at 4˚C once thawed.

10. SAMPLE PREPARATION

10.1. **Cell Extracts:**

Adherent cells

10.1.1. Remove media and wash cells with PBS.

10.1.2. Place the culture plate on ice, add cold Lysis Buffer and keep the plate on ice for one minute.

10.1.3. Scrape the cells and gently transfer the disrupted cell suspension into a chilled microcentrifuge tube.

10.1.4. Mix on a rotary mixer for 30 minutes at 4°C.

10.1.5. Centrifuge at 10,000 x g for 10 minutes at 4°C and transfer the cell extract to chilled fresh tubes.

Suspension Cells

10.1.6. Collect cells by centrifugation, Wash the cells with PBS at room temperature and collect cells again by centrifugation.

10.1.7. Drain the PBS carefully, add cold Lysis Buffer and keep the cells on ice for 1 minute. Follow steps 10.1.4 – 10.1.5.

10.2. **Tissue Extracts:**

10.2.1. Snap freeze the dissected tissue and immediately grind it into a fine powder using a mortar and pestle in a liquid nitrogen bath.

10.2.2. Transfer the ground tissue to a pre-weighed chilled tube. Weigh the powder and store at -80°C until use

10.2.3. Add 300 µL Lysis Buffer with Protease Inhibitor Cocktail I/protease inhibitors per 5 mg of tissue powder. Mix on a rocker at 4˚C for about an hour.

10.2.4. Pass the lysate through a 25 gauge needle 3 times.

10.2.5. Collect the lysate and centrifuge at high speed (10,000 x g) for 5 minutes at 4˚C to remove cell debris.

10.2.6. Transfer the tissue extract (supernatant) to a fresh tube.

11. ASSAY PROCEDURE

11.1. Antibody Binding

11.1.1. Add a predetermined amount (µg) of antibody (as recommended by the antibody vendor or as determined by user titration) against the target to a known amount (µg) of sample (standardized by the user). Make up the volume to 500 µL with Lysis Buffer containing the Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail.

11.1.2. Gently mix for 3-4 hours or overnight at 4°C on a rotary mixer.

11.2. Preparation of Protein A/G Beads

11.2.1. Wash the Protein A/G Sepharose Beads I/Protein A/G Sepharose® (25-40 µL/reaction) twice with 1 mL Wash Buffer X/Wash Buffer, centrifuging at 2000 x g for 2 minutes and aspirating the supernatant in between washes. Note that 25 µL Protein A/G Sepharose Beads I/Protein A/G Sepharose® beads can bind over 500 µg IgG.

11.2.2. Suspend as 50% slurry in 1 X Wash Buffer X/Wash Buffer.

11.3. Bead Capture

11.3.1. After antibody binding (step 11.1), add 25-40 µL of Protein A/G Sepharose Beads I/Protein A/G Sepharose® beads slurry to each tube and Gently mix for 1 hour at 4°C on a rotary mixer.

11.3.2. Collect the Protein A/G Sepharose Beads I/Protein A/G Sepharose® beads by low speed centrifugation at 4°C (e.g., 2000 x g for 2 minutes).

11.3.3. Wash Protein A/G Sepharose Beads I/Protein A/G Sepharose® beads 3 times with 1 mL 1X Wash Buffer X/Wash Buffer, collecting the Protein A/G Sepharose Beads I/Protein A/G Sepharose® beads by low speed centrifugation at 4°C and aspirating the supernatant in between washes.

11.3.4. After the last wash, remove as much of the 1X Wash Buffer X/Wash Buffer as possible, making sure that the beads never dry completely.

11.4. Elution

11.4.1. Functional Assay:

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

11.4.2. SDS Buffer (denaturing) elution:

To elute the complex, add 40 µL 2X SDS-PAGE loading buffer (not provided) to the beads and boil for five minutes. Centrifuge to collect eluent. Eluent can be stored on ice for same day analysis or frozen at -80˚C for future SDS-PAGE analysis.

11.4.3. Low-pH (non-denaturing) elution:

Add 40 µL low pH glycine buffer (100 mM glycine/HCl, pH 2.5-3.0, not provided) and incubate for 10 minutes at room temperature with 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 pH and 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.

12. TYPICAL DATA

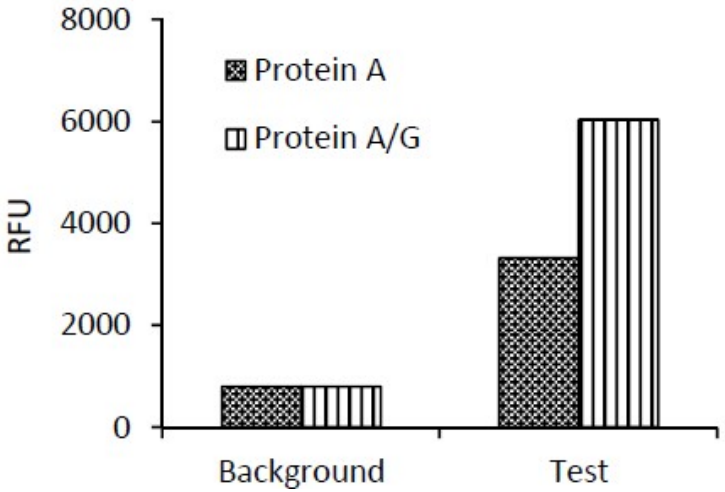


Figure 1: Comparison of immunoprecipitation using Protein A beads and Immunoprecipitation kit ab206996. HDAC2 activity assay of the antigen-antibody complex captured using Protein A beads or ab206996 by following the same protocol demonstrates that ab206996 is more efficient in IP than Protein A beads.

13. NOTES

RESOURCES

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Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

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