Antibody Magnetic Bead IP Kit User Manual

- **Size: 20 Tests/Kit or 50 Tests/Kit**
- **Expiration: See kit label**

Kit Components

Component Name	Storage	20 Tests	50 Tests
Protein Standard (Pos. Control) Positive Lysate (Pos. Control)	-20 °C	20 µL / 1.5 mL	50 µL / 3.5 mL
Antibody Magnetic Beads	2~8 °C	1.2 mL	2.7 mL
Lysis Buffer	2~8 °C	250 mL	500 mL
Loading Buffer A	2~8 °C	3 mL	5 mL
Elution Buffer (pH 2.5)	2~8 °C	5 mL	10 mL
Neutralization Buffer (pH 8.5)	2~8 °C	0.5 mL	1 mL

Note: Reagents and tools required but not provided:

・PBS Buffer (recipe below) ・β-mercaptoethanol ・Magnetic Rack

Reagent Preparation

PBS Buffer (For 1 L)

Component	Quantity
NaCl	8 g
KCl	0.2 g
KH_2PO_4	0.2 g
$Na_2HPO_4 \cdot 12H_2O$	2.9 g
Deionized Water	To1 L

Note: Mix thoroughly until fully dissolved. Use as needed.

Loading Buffer B

Component	Volume
Loading Buffer A	50 µL
β-mercaptoethanol	1 µL

Note: Prepare immediately before use. Mix thoroughly.

Sample Processing

1. Lysis and Preparation of Cell or Tissue Samples

Before lysis, add an appropriate protease inhibitors to the supplied Lysis Buffer. For samples with high nucleic-acid content, a nuclease may be added as required. Keep the prepared lysis buffer on ice until use.

Suspension Cells:

- Collect cells from culture. Centrifuge at 2-8°C, 5000 rpm for 5 min.
- Discard supernatant. Wash cell pellet once with cold PBS Buffer (2-8 ° C). Discard PBS Buffer and remove residual liquid as thoroughly as possible.
- Add Lysis Buffer at a ratio of **100-200 µL per 5-10 million cells** (adjust based on the total protein concentration). Resuspend by gentle pipetting and avoid introducing bubbles. After addition of lysis buffer, animal cells are generally lysed rapidly. Alternatively, incubate on ice for **5–10 min** to ensure complete lysis.

Adherent Cells:

- Remove culture medium. Detach cells by scraping and collect into a centrifuge tube.
- Wash the culture vessel with cold PBS Buffer 3 times. Collect the wash and pellet cells by centrifugation.
- Proceed with centrifugation and lysis as described for Suspension Cells.

Tissue Samples:

- Frozen tissue samples may be thawed on ice. Mince fresh or thawed tissue into small pieces.
- Add Lysis Buffer at a ratio of **100-200 µL per 10-20 mg tissue** (adjust based on the total protein concentration and tissue lysis efficiency). Homogenize thoroughly using a homogenizer or tissue grinder until the tissue is completely disrupted. For fibrous or hard tissues, snap-freeze in liquid nitrogen and grind to a fine powder in a mortar before adding Lysis Buffer. Incubate on ice for **5-10 min** to ensure complete lysis.

2. Sonication (Optional)

- After addition of lysis buffer, animal cells are generally lysed within **1–3 s**. If obvious clumps remain after ice-bath lysis, incomplete lysis is suspected and sonication may be considered.
- Aliquot lysate into 2 mL microcentrifuge tubes (0.5-1.2 mL per tube). Sonicate at **60% power, 5 cycles of 2 s on / 20 s off**.
- After sonication, examine lysis efficiency under a microscope. Most cell/tissue clumps should be disrupted.

3. Centrifugation

- Centrifuge the lysate at **2-8° C , 15000 rpm for 10 min**. Transfer the supernatant to a pre-cooled tube. This is the **Lysate**.

4. BCA Quantification (Optional, adjust based on needs)

- Dilute the Lysate 10-fold with Lysis Buffer.
- Measure total protein concentration using the BCA method.
- Dilute the Lysate with Lysis Buffer to a final concentration of **1 mg/mL** for the IP experiment.

Note: If BCA quantification is skipped, skip the steps above and use the Lysate obtained in Step 3 directly for subsequent experiments.

Antibody Beads & Immunoprecipitation Binding

1. Bead Preparation

- Thoroughly resuspend Antibody Magnetic Beads to a uniform suspension.
- Transfer **50 µL** of Antibody Beads to a tube.
- Add **1 mL Lysis Buffer**. Mix gently by inversion.
- Place on a magnetic rack to separate. Discard supernatant.
- Repeat this wash step **3 times**.

2. Immunoprecipitation (IP)

- Add **200 µg** of cell/tissue lysate (approx. 200 µL if 1 mg/mL) to the prepared Antibody Beads.
- If protein concentration is high, dilute with Lysis Buffer to **1 mg/mL**.
- Incubate with gentle rotation at **2-8°C overnight**.

Note: Bead input and incubation time may be adjusted based on experimental purpose and pilot results. Overnight incubation at 2-8° C is recommended. Incubation may be shortened or performed at room temperature for a shorter duration as needed.

- Positive Control (Optional): Can be prepared using either method below:

  A. Positive Protein Standard: Add 20 µL Protein Standard to 200 µL Lysis Buffer, then add to prepared beads. Incubate 2-4h at 2-8°C, or overnight.

  B. Positive Lysate: Add 200 µL Positive Lysate to prepared beads. Incubate 2-4h at 2-8°C, or overnight.
- After incubation, place the tube on the magnetic rack. Transfer the supernatant (flow-through) to a new tube (optional, for analysis). The immune complex is now bound to the beads.
- Add **1 mL Lysis Buffer** to the bead-complex. Mix gently by inversion. Place on magnet to separate. Discard supernatant. Repeat this wash step **3-6 times**.

3. Elution

Note: Select the appropriate elution method according to experimental requirements. Among the two methods below, Method B provides higher elution efficiency, whereas Method A results in lower antibody dissociation.

A. Acid Elution (Non-denaturing)

This method is non-denaturing. The antibody remains bound to the beads, and the eluate can be used for functional assays.

- Add **20 µL Elution Buffer** to the antibody-bound beads and shake at room temperature for **5 min**.
- Place the tube on a magnetic rack to separate the beads. Collect the supernatant into a new microcentrifuge tube.

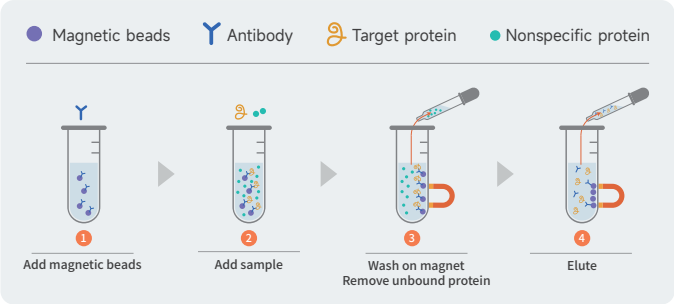
- Add **2 µL Neutralization Buffer** to adjust the pH to approximately **7.4**.
- Repeat the elution once more and combine the two eluates as the final elution product.

Note: If SDS-PAGE analysis is required, mix a portion of the eluate with an equal volume of Loading Buffer B, mix thoroughly, and heat at high temperature prior to electrophoresis.

B. Reducing Elution (Denaturing)

This method is denaturing and provides higher elution efficiency. Mild antibody dissociation may occasionally occur.

- Place the tube on a magnetic rack to separate the beads and discard the supernatant.
- Add **30 µL Loading Buffer B** to the beads and resuspend thoroughly by pipetting.
- Heat at **95°C** using a metal heating block or by boiling.
- Briefly centrifuge using a benchtop centrifuge and collect the supernatant as the final elution product.



Precautions

- When performing immunoprecipitation, it is recommended to include an immunoglobulin control of the same species origin, isotype, and dosage as the primary antibody to assess potential non-specific binding.
- Low-adsorption microcentrifuge tubes and pipette tips are recommended to minimize sample loss caused by nonspecific adsorption to consumables.
- Do not subject magnetic beads to excessive centrifugation, drying, or freezing. Such treatments may lead to bead aggregation and loss of binding activity.
- When magnetic separation is not required, avoid leaving the beads on the magnetic rack for prolonged periods.
- Increased bead stickiness or occasional aggregation after antibody coupling is normal. Aggregates may be dispersed by pipetting, gentle vortexing, or low-power sonication. Minor aggregation generally does not affect immunoprecipitation performance.
- Use clean laboratory consumables free of exogenous proteins to prevent contamination.