Product Specification

Product Name: Smart Streptavidin Magnetic IP/Co-IP Kit

Product Code: MAGP101-K10T / MAGP101-K50T

PRODUCT DESCRIPTION:

The **Smart Streptavidin Magnetic IP/Co-IP Kit** is an advanced system designed for high-efficiency immunoprecipitation (IP) and co-immunoprecipitation (Co-IP) experiments. It features Streptavidin MagPoly Beads that facilitate fast and effortless magnetic separation, combined with optimized buffers to ensure stable and consistent experimental reactions. The beads are coupled with recombinant streptavidin subunits (~14 kDa), which provide exceptionally high affinity for biotinylated antibodies while minimizing non-specific background noise due to the lack of carbohydrate groups. This versatile kit is compatible with a wide range of biological sample types, including serum, ascites fluid, cell lysates, and cell culture supernatants.

Components of Smart Streptavidin Magnetic IP/Co-IP Kit

Component Name	MAGP101-K10T (10T)	MAGP101-K50T (50T)
Streptavidin MagPoly Beads	200µl	1ml
IP Lysis/Wash Buffer (5×)	10ml	25ml × 2
IP Lysis/Wash Buffer Enhanced	100µl	500µl
IP Elution Buffer	500µl	1ml × 5
Neutralization Buffer	2ml	2ml

PRECAUTIONS

Preparation: Review this manual thoroughly before starting. Unless otherwise specified, perform all steps at 4°C.

Buffer Selection: While the 1× IP Lysis/Wash Buffer (Enhanced) provides effective washing, it may diminish binding affinity. For standard washing steps, using the regular 1× IP Lysis/Wash Buffer is recommended.

Bead Handling: Keep magnetic beads in storage solution to avoid desiccation; resuspend completely before use. Note that boiled beads aggregate and lose binding capacity; they must be discarded.

Elution: For reducing conditions, supplement the SDS-PAGE loading buffer with DTT for a final concentration of 10 – 20mM.

Optimization: Use high-specificity antibodies for best results. The binding affinity between antibodies and antigens varies depending on the antibody type and can be affected by experimental conditions. If the provided buffers underperform, optimize the buffer conditions to suit your specific antibody-antigen pair.

Quality Control: Always include control groups and retain fractions from each incubation step for troubleshooting and verification.

OPERATION PROCEDURE

1. Buffer Preparation

Depending on your experimental needs, you can opt for the buffers in the kit or develop your own buffer systems. The IP Lysis/Wash Buffer (5×) should be diluted with pure water before use and labeled as 1× IP Lysis/Wash Buffer. Alternatively, it can be supplemented with IP Lysis/Wash Buffer Enhanced to a final concentration of 0.1% – 1% and labeled accordingly as 1× IP Lysis/Wash Buffer (Enhanced). **All buffers are recommended to be filtered through a 0.22μm or 0.45μm filter prior to use.** Diluted buffers should be stored at 4°C. Any reagents that turned cloudy should be discarded immediately.

The following are additional materials that are required but not provided and should be **prepared beforehand**:

- **Non-reducing SDS-PAGE loading buffer (5×):** 0.3 M Tris-HCl, pH 6.8, 5% SDS, 50% glycerol, 0.5% bromophenol blue
- **Dithiothreitol (DTT)**
- **Protease inhibitor**
- **The antigen and antibody to be used for the immunoprecipitation**

2. Sample Preparation

2.1 Protocol I: Lysis of Adherent Cells

1) Carefully aspirate the culture medium from the adherent cells.

2) Wash the cells twice with ice-cold PBS.

3) Add the recommended volume (see Table below) of pre-chilled 1× IP Lysis/Wash Buffer (Enhanced).

4) Incubate on ice for 5 minutes, agitating the plate gently several times.

5) Transfer the lysate to a new microcentrifuge tube and centrifuge at approximately 13,000 × g for 10 minutes to pellet the cellular debris.

6) Transfer the supernatant to a fresh tube and label as 'cell lysate'. This sample is ready for protein quantification and downstream applications.

Recommended Volume of 1x IP Lysis/Wash Buffer (Enhanced) for Various Standard Culture Plates

Culture Plate Size/Surface Area	Volume of IP Lysis/Wash Buffer
100 x 100mm	500 - 1000µl
100 x 60mm	250 - 500µl
6-Well Plate	200 - 400µl per Well
24-Well Plate	100 - 200µl per well

2.2 Protocol II: Lysis of Cell Suspension Cultures

1) Pellet the cell suspension by centrifuging at 1,000 × g for 5 minutes, then discard the supernatant.

2) Wash the cells by gently resuspending the pellet in ice-cold PBS; centrifuge again at 1,000 × g for 5 minutes and remove the supernatant.

3) Lyse the cells by adding pre-chilled 1× IP Lysis/Wash Buffer (Enhanced) at a ratio of 500 µL per 50 mg of cell pellet.

4) Incubate on ice for 5 minutes, agitating periodically.

5) Clarify the lysate by centrifuging at 13,000 × g for 10 minutes to remove debris.

6) Collect the resulting supernatant into a fresh tube; label this "cell lysate" for protein quantification or downstream assays.

3. Immunoprecipitation Process

The sequence for binding the antigen, antibody, and magnetic beads should be optimized based on your practical requirements. Since the incubation order directly impacts antigen recovery and purity, we have outlined a standard and most adopted approach.

3.1 Magnetic Bead Wash

1) Resuspend the Streptavidin MagPoly Beads completely and transfer 20 µl (0.2 mg) into a 1.5 ml microcentrifuge tube.

2) Add 180 µl of 1× IP Lysis/Wash Buffer (Enhanced) and vortex briefly.

3) Place the tube on a magnetic stand. Once the solution clears, carefully remove and discard the supernatant.

4) Add 1 ml of the 1× IP Lysis/Wash Buffer (Enhanced), then either invert the tube repeatedly or vortex for 1 minute.

5) Place the tube on the magnetic stand again and discard the supernatant once the beads have fully sequestered.

3.2 Immunoprecipitation

3.2.1 Protocol I

1) Add 2 – 10 µg of antibody to the pre-washed magnetic beads (from Step 3.1). Bring the final volume to 500 µl using either the antibody storage buffer or 1× IP Lysis/Wash Buffer.

2) Incubate the mixture at room temperature for 30 minutes with continuous agitation. Use a magnetic stand to sequester the beads. Retain the supernatant for downstream analysis.

   Note: Incubation times may vary from 30 minutes to 2 hours at room temperature, or for 1 to 16 hours at 4°C, depending on the actual binding efficiency. Adjust accordingly.

3) Resuspend the beads in 500 µl of 1× IP Lysis/Wash Buffer. Mix via inversion or gentle vortexing for 1 minute, then use the magnetic stand to sequester the beads and discard the supernatant. Repeat this wash at least twice.

4) Add 500 µl of the cell lysate from Step 2 (containing 500 – 1000 µg of total protein) to the bead-antibody complex. If necessary, use 1× IP Lysis/Wash Buffer (enhanced) to achieve the total volume of 500 µl. Incubate at room temperature for 30 minutes with vortexing, then use the magnetic stand to sequester the beads. Retain the supernatant for subsequent analysis.

   Note: Incubation times may vary from 30 minutes to 2 hours at room temperature, or for 1 to 16 hours at 4°C, depending on the actual binding efficiency. Adjust accordingly.

3.2.2 Protocol II

1) Add the cell lysate (from Step 2) to 2 – 10 µg of antibody and incubate for 30 minutes. Each reaction should ideally contain 500–1000 µg of total protein; if the volume is less than 500 µl, supplement it with 1× IP Lysis/Wash Buffer (Enhanced).

2) Transfer this mixture to the magnetic beads from Step 3.1 and incubate with continuous mixing.

   Note: Incubation times may vary from 30 minutes to 2 hours at room temperature, or for 1 to 16 hours at 4°C, depending on the actual binding efficiency. Adjust accordingly.

3) Place the tube on a magnetic rack. Once the beads have sequestered, retain the supernatant for further analysis.

3.3 Magnetic Bead Wash

1) Resuspend the beads in 500 µl of 1× IP Lysis/Wash Buffer (Enhanced) by inverting the tube or vortexing gently for 1 minute.

2) Place the tube on a magnetic rack. Once the beads are fully sequestered, aspirate and discard the supernatant. Repeat this wash once.

3) Add another 500 µl of the 1× IP Lysis/Wash Buffer (Enhanced) buffer to the beads, then transfer the entire suspension to a fresh microcentrifuge tube. Mix by inversion or gentle vortexing for 1 minute.

4) Return the tube to the magnetic rack, allow the beads to sequester, then discard the supernatant.

3.4 Elution

3.4.1 Protocol I: Low-pH Elution

1) Resuspend the beads in 50 µl of IP Elution Buffer and incubate at room temperature for 10 minutes with continuous mixing.

2) Use a magnetic rack to clear the beads, then recover the supernatant.

3) Add 5 - 10µl of Neutralization Buffer to the eluate.

3.4.2 Protocol II: Alternative Elution Method (Denaturing Elution)

1) Resuspend the beads in 50 µl of 1× SDS-PAGE loading buffer and heat at 96–100°C in a water bath for 10 minutes.

2) Use a magnetic stand to sequester the beads, then recover the buffer containing the denatured target antigen.

Note: Both elution strategies recover the antibody-antigen complex. However, while the Low-pH Elution method preserves the antibody's integrity, the Denaturing Elution method causes it to dissociate into its heavy and light chains. Select the method that is best suited for your specific downstream applications.

TROUBLESHOOTING

Problem	Possible Cause	Solution
Antigen was not immunoprecipitated	Insufficient antigen in the starting sample.	Validate protein levels via SDS-PAGE / Western Blot and increase the initial antigen amount to the recommended level.
	Weak antibody binding affinity	Optimize the Lysis/Wash Buffer; Replace the antibody with one that has higher affinity / specificity, or with one that recognizes a different epitope.
	Protein degradation	Add protease inhibitors.
Antigen is absent in the elution fraction	Low Expression Levels	Optimize the expression conditions.
	Elution conditions are too mild	Extend the elution incubation time or switch to a stronger elution buffer.
Eluted antibody bands interfere with antigen detection	Antigen size is close to 25 kDa or 50 kDa	Avoid reducing the sample before SDS-PAGE to shift the antibody bands to ~160 kDa; For Western blotting, use secondary antibodies derived from a different species than the primary antibody.
Non-specific bands are prominent	Non-specific protein binding to magnetic beads	Optimize the composition of the wash buffer (e.g., supplementing with 50–350mM NaCl).

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