Instruction Manual

His Tag Immunoprecipitation Kit

Introduction

TargetMol's His Tag Immunoprecipitation Beads specifically bind to His-tagged proteins and are suitable for immunoprecipitation (IP) of proteins, protein complexes, protein–nucleic acid complexes, and other antigens. The IP kit includes optimized, ready-to-use buffers that provide ideal reaction conditions for immunoprecipitation, enhancing the stability and consistency of the experiment. This product is suitable for antigens from cell lysates, cell culture supernatants, serum, ascitic fluid, and other biological samples.

Product Features

- Low non-specific binding
- Time saving and efficient usage
- Convenient and simple operation
- Assay consistency

Product Components

Catalog No.	Product Name	Package Size
C0123B	His Tag Immunomagnetic Beads	1 mL
C0124	Immunomagnetic Beads Cell Lysis Buffer	20 mL
C0125	Immunomagnetic Beads Washing Buffer (10×)	20 mL
C0126	Immunomagnetic Beads Elution Buffer	4 mL
C0127	Immunomagnetic Beads Neutralization Buffer	2 mL

His Tag Immunomagnetic Beads	Specification
Matrix	Silica-based magnetic beads
Particle Size	200 nm
Binding Capacity	≥ 0.5 mg His-tagged protein/mL beads
Bead Concentration	10 mg/mL
Ligand	Mouse-derived anti-His monoclonal antibody
Recommended Applications	IP, Co-IP

Instruction

1. Preparation of Cell Lysates

Use Cell Lysis Buffer to process cell samples. Prepare cell lysates following standard procedures, keep the lysates on ice for immediate use, or store at –20 °C for long-term storage.

2. Pretreatment of Magnetic Beads

1) Vortex the immunoprecipitation magnetic beads for 1 minute to fully resuspend. Transfer 25 µL of bead suspension into a 1.5 mL microcentrifuge tube.

2) Dilute Washing Buffer (10×) with distilled water to prepare a 1× solution. Use Washing Buffer (1×) for subsequent experiments. Add 500 µL of Washing Buffer into microcentrifuge tube to wash the beads. Gently invert the tube several times to resuspend the beads. Place the tube in a magnetic separator for 1 minute, then remove and discard the supernatant. Repeat the washing step twice.

3. Immunoprecipitation

1) Add 500 µL of prepared cell lysate to a microcentrifuge (EP) tube. Place the tube on a rotator and mix at 37 °C for 30 minutes. For weaker interactions, incubate for 1 hour at room temperature or overnight at 4 °C.
2) After incubation, perform magnetic separation. Discard or retain the supernatant for further analysis as needed.
3) Add 500 µL of Washing Buffer to the tube to wash the beads. Perform magnetic separation and discard the supernatant. Repeat the washing step 3 times.

4. Elution of Target Proteins

Three elution methods are provided below.

1) Denaturing Elution: Suitable for SDS-PAGE analysis. Add 100 µL SDS-PAGE Loading Buffer (user-supplied) to the tube, mix well, and heat at 95 °C for 5 minutes. Then perform magnetic separation or centrifuge (13,000 g, room temperature, 10 min), and collect the supernatant for SDS-PAGE.
2) Neutral Elution Method: Add 50 µL of His Peptide Elution Buffer (PBS, 1 mg/mL 6x His peptide [TP1280], pH 7.4) to the EP tube. Incubate on a rotator at 37 °C for 5–10 minutes (extend the incubation time if the temperature is below 37 °C). Then perform magnetic separation or centrifugation and collect the supernatant. To improve antigen recovery, the elution step can be repeated.
3) Acidic Elution: Add 100 µL of Acidity Elution Buffer to the tube. Incubate on a rotator at 37 °C for 5–10 minutes. Then perform magnetic separation or centrifugation, and collect the supernatant. To neutralize the eluate, add 50 µL of Neutralization Buffer to 100 µL of eluate to adjust the pH to neutral.

Storage

C0124: Store at -20℃ for 2 years.
Other reagents: Store at 4℃ for 2 years.

Precautions

1. Avoid freezing the beads. Store in solution to prevent drying.
2. The average magnetic separation time should be longer than 1 min.
3. Ensure uniform suspension by fully shaking the storage tube before use. Avoid bubbles during operation.
4. It is recommended to use high-quality pipette tips and reaction tubes to reduce bead and solution loss due to surface adhesion.
5. Use high-quality tips and test tubes to avoid sample loss due to adhesion.
6. In IP experiments, the binding affinity of different proteins may vary. Users can select and prepare buffers according to experimental needs.
7. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
8. Please wear a lab coat and disposable gloves.