

Flag Tag Immunomagnetic Beads

Introduction

TargetMol's Flag Tag Immunomagnetic Beads specifically bind to proteins with the DYKDDDDK (Flag) tag, which can be used for immunoprecipitation (IP) of proteins, protein complexes, protein-nucleic acid complexes, and other antigens. This product is suitable for antigen samples from cell lysates, cell culture supernatants, serum, ascites, etc.

Product Features

1. Low non-specific binding
2. Time saving and efficient usage
3. Convenient and simple operation
4. Assay consistency

Product Information

Flag Tag Immunomagnetic Beads	
Material	Silica-based magnetic beads
Bead Size	200 nm
Concentration	10 mg/mL
Binding Capacity	≥ 0.6 mg Flag tag protein/mL beads
Ligand	Anti-Flag monoclonal antibody produced in mouse
Recommended Applications	IP, Co-IP

Instructions

Prepare Reagents

Reagent	Formulation
Washing Buffer (1×)	TBST: 50 mM Tris-HCl, 150 mM NaCl, 0.1%(v/v) Tween-20, pH7.4
Flag Peptide Elution Buffer	PBS, 1 mg/mL 3× Flag peptide (TP1274), pH 7.4
Acidity Elution Buffer	0.1 M Glycine, 0.1% (v/v) Tween-20, pH2.5
Neutralization Buffer	1 M Tris-HCl, pH 9.0

Preparation of cell lysates

Select an appropriate lysis buffer to lyse cell samples and obtain cell lysates. Place on ice or store at -20 °C for long-term use.

Pretreatment of Magnetic Beads

- (1) Vortex for 1 min to resuspend the immunomagnetic beads. Take 25-50 µL of suspension and place it in a 1.5 mL EP tube.
- (2) Add 500 µL of Washing Buffer to the EP tube and gently invert several times to resuspend the beads. Keep the EP tube in a magnetic separator and stand for 1 min for magnetic separation. Finally, remove the supernatant and then take off the EP tube. Repeat the washing steps twice.

Immunoprecipitation

- (1) Add 500 µL of prepared cell lysates to the EP tube. Place it on a rotating mixer and rotate at 37 °C for 30 min. For weak binding, incubate at room temperature for 1 hour or overnight at 4 °C.

- (2) After incubation, perform magnetic separation, then remove or save the supernatant for further analysis.
- (3) Add 500 μ L of Washing Buffer to the EP tube. Perform magnetic separation. Finally, remove the supernatant and then take off the EP tube. Repeat the washing steps 3 times.

Elution of Target Proteins

- (1) Denaturing Elution: Suitable for SDS-PAGE detection. Add 100 μ L of SDS-PAGE Loading Buffer to the EP tube. Mix well and heat at 95 $^{\circ}$ C for 5 min. Perform magnetic separation or centrifugation (room temperature, 13000 g, 10 min) to collect the supernatant.
- (2) Neutral Elution: Add 50 μ L of Flag Peptide Elution Buffer to the EP tube. Incubate on a rotating mixer at 37 $^{\circ}$ C for 5-10 min (longer incubation time when below 37 $^{\circ}$ C). Then perform magnetic separation or centrifugation to collect the supernatant.
- (3) Acidity Elution: Add 100 μ L of Acidity Elution Buffer to the EP tube. Incubate on a rotating mixer at 37 $^{\circ}$ C for 5-10 min. Perform magnetic separation or centrifugation to collect the supernatant. To adjust the pH of acidic elution buffer to neutral, add 50 μ L of Neutralization Buffer to 100 μ L elution.

Storage

Store at 4 $^{\circ}$ C 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. Use high-quality tips and test tubes to avoid sample loss due to adhesion.
5. Test the binding of proteins to beads by using the collected supernatant.
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.

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