Anti-His Immunomagnetic Beads

（CZ010）

Product Overview

A His-tag peptide typically consists of 6 or 8 histidine residues, which can be fused to the N-terminus or C-terminus of a fusion protein. Owing to its small molecular weight and ease of separation and purification, the His-tag fusion tag has distinct advantages over other tags and is currently the most widely used fusion tag for protein purification. The His-tag peptide can specifically bind to nickel ions, and recombinant proteins with a histidine tag can be adsorbed by nickel columns to achieve purification. Anti-His antibodies can be used to detect the expression and intracellular localization of His-tagged fusion proteins, as well as for the purification, qualitative and quantitative detection of such proteins. They are suitable for protein-protein interaction experiments such as Western Blot (WB) and Immunoprecipitation (IP).

Components

Cat. No.	Product Name	Volume
CZ010	Anti-His Immunomagnetic Beads	1 mL

Transportation and Storage

Transport with ice packs. Store at 4℃.

Notes

1. Slight agglomeration of the product is a normal phenomenon and does not affect normal use.
2. Do not use cell lysate samples containing reducing agents such as DTT with this product, as DTT may cause the detachment of antibody ligands.
3. This product can tolerate urea at a concentration of ≤2 M; high concentrations of urea will lead to the detachment of antibody ligands.
4. Avoid aspirating the magnetic beads during washing and elution. Aspirating beads during washing will affect the final loading capacity, and aspirating beads during elution will impair the quality of the target protein and electrophoresis results. After the first elution, transfer the eluate to a new centrifuge tube and place it on a magnetic stand for re-adsorption.

Usage Method

This protocol is mainly for protein-protein interaction experiments, with 20 μL of Anti-His Immunomagnetic Beads used per reaction.

1. Buffer Preparation (Prepared by the user)

Buffer	Reagent Formulation
Wash Buffer	20 mM Na_2HPO_4,0.15 M NaCl,0.05% Tween-20,pH=7.4
Elution Buffer	0.1M Gycine,0.15M NaCl,pH=3.0

Neutralization Buffer	1 M Tris-HCl, pH 8.0

2. Bead Rinsing

   (1) Resuspend the magnetic beads thoroughly, aliquot 20 μL of beads into a centrifuge tube, add 500 μL of Wash Buffer, and invert the tube 5-10 times to mix well.

   (2) Place the centrifuge tube on a magnetic stand, and aspirate the supernatant after all beads are adsorbed.

   (3) Add another 500 μL of Wash Buffer to the tube, invert 5-10 times to mix well, place on a magnetic stand, and aspirate the supernatant after all beads are adsorbed.

   (4) Repeat the above operation 3 times.

3. Sample Incubation

   (1) Add 500-1000 μL of cell lysate sample to the rinsed beads, and incubate at room temperature or 4℃ for 60 minutes (incubation on a rotator is recommended to ensure full contact between the sample and beads).

   (2) After incubation, place the centrifuge tube on a magnetic stand; after all beads are adsorbed, transfer the supernatant to a new centrifuge tube and retain it for subsequent detection.

   (3) Add 500 μL of Wash Buffer to the tube, invert gently 5-10 times to mix well, place on a magnetic stand, and discard the supernatant after all beads are adsorbed.

   (4) Repeat the above operation 5 times.

   Note： The recommended incubation conditions are 0.5-2 hours at room temperature or 1-4 hours at 4℃; adjust the incubation time appropriately according to the actual binding efficiency. Overnight incubation is generally not recommended, as it may cause excessive non-specific adsorption. For each bead washing step, the mixture can be incubated on ice for 3-5 minutes after adding the Wash Buffer.

4. Protein Elution

   (1) Elution with Elution Buffer: Add 40 μL of Elution Buffer per 20 μL of the original bead volume, elute at room temperature or 4℃ for 5-10 minutes, and mix gently 3-5 times during elution. After incubation, place the centrifuge tube on a magnetic stand; after all beads are adsorbed, aspirate the supernatant to a new centrifuge tube, add a small amount of Neutralization Buffer to adjust the pH to neutral, and perform Western Blot detection finally.

   (2) Elution with SDS-PAGE Loading Buffer: Add 60 μL of 1×SDS Loading Buffer per 20 μL of the original bead volume, mix well by gentle shaking, and heat at 95-100℃ for 5-10 minutes. Place the centrifuge tube on a magnetic stand for 10 seconds for separation, and collect the supernatant for SDS-PAGE electrophoresis or Western Blot detection.

Frequently Asked Questions and Answers

1. What is the difference between polymer magnetic beads and agarose magnetic beads?

   (1) Agarose magnetic beads are porous microspheres with a particle size of 30-100 μm. Some ligands will enter the pores during cross-linking, resulting in a relatively high coupling capacity, making them suitable for rapid protein purification.

Polymer magnetic beads usually have a particle size of less than 1-3 μm, with ligands mainly distributed on the microsphere surface. This type of beads can bind proteins rapidly with minimal steric hindrance, making them suitable for protein-protein interaction experiments, but their loading capacity is relatively low.

(2) Agarose magnetic beads are recommended for protein purification experiments, while polymer magnetic beads are recommended for protein-protein interaction experiments such as IP, Co-IP and pull-down assays.

2. Why is the target protein not captured?

(1) The failure may be caused by the absence of target protein expression. It is recommended to detect the original expression level of the protein by WB before purification; if there is no WB signal, it will be difficult to capture the target protein.

(2) There are interfering substances such as high-concentration reducing agents in the sample. It is recommended to select an appropriate lysis buffer, or directly use sonication for cell lysis (sonication is not recommended for Co-IP experiments).