Anti-DYKDDDDK (Flag) Magnetic Beads

Introduction

The Flag tag is a polypeptide consisting of 8 amino acid residues (DYKDDDDK), usually in the form of Flag or 3X Flag. It can be linked to the 5' or 3' end of the gene of interest through genetic recombination technology to form a target protein with a Flag tag. Flag tags are widely used in the study of protein expression, purification, identification, interaction and function because they do not interact with the target protein and do not affect the function of the target protein, the N-terminal Flag tag can be excised by enterokinase to obtain an untagged target protein, and can be detected and purified by Flag antibody, Anti-Flag magnetic beads or Anti-Flag affinity gel.

Anti-DYKDDDDK (Flag) Magnetic Beads is a biological experiment tool designed for life science research. It is composed of a high-quality Flag monoclonal antibody covalently conjugated to nanoscale amino magnetic beads, which is immobilized on the surface of the beads to specifically bind to the protein containing the Flag tag, and then through magnetic separation technology, the target protein can be rapidly purified and detected.

This product can be used for immunoprecipitation (IP), co-immunoprecipitation (Co-IP), protein purification, protein expression and functional research. Available in 1 mL and 2 mL formats.

Product parameters

Coupled antibody	Anti-Flag Tag monoclonal antibody
Beads size	1 μm
Concentration	10 mg/mL
Binding capacity	≥ 0.6 mg DYKDDDDK-tagged fusion protein/mL of beads.
Application	IP、Co-IP、DYKDDDDK (Flag)-tagged fusion protein purification

Compositions and storage

Components	1 mL	2 mL
Anti-DYKDDDDK (Flag) Magnetic Beads	1 mL	2X1 mL
Store the components at 4°C for 12 months. Please avoid freezing storage.		

Protocol

1. Self-supplied reagents

- Bind/Wash buffer: 50 mM Tris, 150 mM NaCl, 0.1%~0.5% detergent (TritonX-100, Tween 20 or NP40), pH 7.5。
- Elution buffer (One chosen): 3X FLAG Peptide (Cat. #: A6001) for competitive elution and 5X Protein Loading Buffer (Reducing) (Cat. #: K1164), Acidic elution is recommended at 0.1 M to 0.2 M Glycine, 0.1% to 0.5% detergent, pH 2.5-3.1 (or 0.1 M citric acid, 0.1% to 0.5% detergent, pH 2.5-3.1).
- Neutralizing buffer (For acidic elution): 1 M Tris, pH 8.0。

2. Preparation of samples

- Adherent cells: Remove the medium and use 150 μL per 1.0×105 cells with 1×PBS (Cat. #: H2018 Wash twice; The cells were scraped off with a cell scraper (or collect cells after normal digestion), collected into a 1.5 mL centrifuge tube, and the binding/washing buffer was added at a ratio of 20~30 μL per 1.0×105 cells, and protease inhibitors were added at the same time, and then incubated on ice for 10 min. Collect the supernatant by centrifugation (4 °C, 14,000 ×g, 10 min) and place on ice for later use (or store at -20 °C for a long time);
- Cells in suspension: Cells were collected by centrifugation (4°C, 500×g, 10 min), discarded and weighed, and washed twice in 1× PBS (Cat. #: H2018) at a ratio of 50 μL per milligram of cells; Binding buffer was added at a ratio of 5~10 μL per milligram of cells, protease inhibitors were added at the same time, mixed well and incubated on ice for 10 min. Collect the supernatant by centrifugation (4 °C, 14,000 ×g, 10 min) and place on ice for later use (or store at -20 °C for a long time);
- Escherichia coli: Collect E. coli (4°C, 12,000×g, 2 min) by centrifugation, discard the supernatant, weigh it, and wash it twice with 1× PBS at a ratio of 10 mL per gram of bacteria (wet weight); Binding buffer was added at a ratio of 5~10 mL per gram of bacteria (wet weight), protease inhibitors were added at the same time, the cells were resuspended, the cells were lysed by ultrasonication, and the supernatant was collected by centrifugation (4°C, 12,000×g, 10 min).

3. Anti-DYKDDDDK (Flag) Magnetic Beads preparation

Because Anti-DYKDDDDK (Flag) Magnetic Beads are stored in a special protective solution, they need to be properly washed before adding to the sample.

a. Gently blow the suspension Anti-DYKDDDDK (Flag) Magnetic Beads with a pipette and take 25μL of the suspension into a clean 1.5mL centrifugation tube.
b. Add 500 μL binding/washing buffer, gently blow with pipette and fully re-suspend Anti-DYKDDDDK (Flag) Magnetic Beads. Place on magnetic rack and separate for 1 min to remove supernatant.
c. Repeat step (3.b) 1-2 time(s) above.

4. Immunoprecipitation, IP

a. Add 500 μL of the sample prepared in step 2 to the pretreated magnetic beads and incubate on an inverted mixer (room temperature for 1 h, 4°C for 4~6 h or overnight);

b. The mixture was placed on a magnetic stand for 1 min, and then the supernatant was transferred to a new centrifuge tube for later use (the supernatant can be used to detect the presence of residues of the DYKDDDDK-tagged protein), and the remaining protein and magnetic bead complexes in the original centrifuge tube are the protein-magnetic bead complexes.

5. Wash

a. In step (4.b), 500 μL of binding/washing buffer was added to the complex species, resuspended by gentle pipette, and then placed on a magnetic stand for 1 minute to remove the supernatant.

b. Repeat the previous step 3 times until the OD280 of the washed supernatant is less than 0.05.

***Note:** If the supernatant OD280 is greater than 0.05, the number of washes should be increased appropriately.

6. Elution

According to the characteristics of the tagged protein and the requirements of subsequent experiments, one of the following three methods can be selected for elution.

- **3X Flag competitive elution method**: This method is a non-denaturing method, with high elution efficiency, and the eluted protein retains the original biological activity, which is convenient for subsequent analysis and detection.

  a. Preparation of 3X Flag Peptide Eluent: Dissolve an appropriate amount of 3X Flag Peptide (A6001) in 1X TBS to a final concentration of 150 μg/mL.

  b. Add 100 μL of 3X Flag peptide eluent (150 μg/mL) per 10-20 μL of raw bead volume, mix well, place on a side swinger or rotary mixer, and incubate for 30–60 minutes at room temperature with shaking, or 1–2 h at 4°C. To improve elution efficiency, the incubation time can be extended, or the elution can be repeated.

  c. After the incubation, place on a magnetic stand for 1 minute and transfer the supernatant to a new centrifuge tube. The supernatant is the eluted Flag-tagged protein.

  d. The eluted Flag-tagged protein is stored at 4°C or -20°C or -80°C for long-term storage.

- **Acid elution method**: This method is a non-denaturing method, which is relatively fast and efficient. The eluted protein retains its original biological activity, which is convenient for subsequent functional analysis and detection.

  a. Add 50–100 μL of acidic eluate to the washed protein-bead complex species and incubate for 5–10 minutes at room temperature, but not more than 15 minutes.

  b. After the incubation, the tubes were separated by static on a magnetic stand for 1 minute, and the supernatant was transferred to a new tube.

  c. 10 μL of neutralization buffer was added per 100 μL of eluent, and the pH of the eluate was adjusted to neutral for later functional analysis.

  d. Elute and neutralize Flag-tagged proteins and store at 4°C for later use, or at -20°C or -80°C for long-term storage.

*Note:

1. Acid elution, while efficient, may still be inferior to competitive elution or SDS-PAGE loading buffer elution.
2. Since the difference of the target protein may have a certain impact on the elution efficiency of the acid eluent method, if the requirements for the elution efficiency are relatively high, the pH of the acid eluent can be adjusted between 2.5-3.1, and the pH value or amount of the corresponding neutralization solution should also be adjusted to a certain extent.

- **SDS-PAGE loading buffer elution method**: This method is a denaturing method, and the obtained protein samples are suitable for SDS-PAGE electrophoresis or WB detection.

  a. Preparation of SDS-PAGE Loading Buffer: Prepare the prepared SDS-PAGE Loading Buffer with water to make 1X. The loading buffer contains a reducing agent such as DTT, and the eluted protein sample will contain the light and heavy chains of the Flag antibody.

  b. Add 80-100 μL 1X SDS-PAGE loading buffer to the washed protein-magnetic bead complex species and heat at 100℃ for 10 min.

  c. After cooling, it was placed on a magnetic stand for 1 min, and after the beads were adsorbed to the side wall of the centrifuge tube, the supernatant was collected for SDS-PAGE electrophoresis or Western blot detection.

FAQs

1. Avoid bead aggregation

   A: 1. Storage conditions: Magnetic beads should be stored in an environment of 2~8°C; 2. Prevent contamination and drying: avoid contamination during use, and ensure that the beads are not dry; 3. Add detergent: Adding 0.1% of a nonionic detergent (e.g., Triton X-100, Tween-20, or NP-40) to the binding/wash buffer is effective in preventing bead aggregation.

2. Resolve the bead adhesion to the tube wall

   A:1. Use low-adsorption consumables**: When performing bead manipulation, it is recommended to use experimental consumables with low adsorption rate; 2. Add detergent: Add 0.01%~0.1% non-ionic detergent to the buffer to reduce the adhesion of magnetic beads.

3. Antigen not precipitated problem

   A:1. Insufficient antigen content: Ensure that the antigen content in the sample is sufficient, which can be verified by SDS-PAGE or Western blot; 2. Increase the amount of sample: if necessary, increase the amount of sample; 3. Buffer composition interference: Try using other buffers for immunoprecipitation and rinsing.

4. The amount of protein is too low

   A:1. Protein degradation: protease inhibitors are added to prevent protein degradation; 2. Increase the number of magnetic beads: If the amount of Anti-DYKDDDDK immune magnetic beads used is insufficient,

consider increasing the dosage.

5. Non-specific banding issues

A: Non-specific protein binding: Add 50~350mM NaCl to the binding/wash buffer and increase the number and intensity of elution.

Note

1. This product needs to maintain a pH of 6-8 and avoid high-speed centrifugation and drying; Do not leave the beads in a magnetic field for a long time, as this may cause the beads to clump.
2. Before using this product, it should be properly and fully suspended, that is, inverted several times to mix the magnetic beads evenly, and the mixing operation should be gentle, and it should not be violently vortex and shaken, etc., to avoid antibody denaturation.
3. At the time of immunoprecipitation or purification, it is recommended to set up positive and negative control groups.
4. Purification of protein samples should be completed as soon as possible after collection and should always be placed at 4°C or in an ice bath to slow down protein degradation or denaturation.
5. If you use an instrument such as a vacuum pump to aspirate the supernatant, you must pay attention to the suction strength of the vacuum pump to avoid excessive suction and sucking up the accumulated magnetic beads.
6. When the acidic solution is eluted, the beads may aggregate, which is a normal phenomenon and does not affect the normal use of the beads. A nonionic detergent of 0.1% (e.g., Triton X-100, Tween-20, or NP-40) effectively prevents bead aggregation and does not affect the antibody binding efficiency of the beads.
7. This product is for scientific research use only.

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