

Anti-DYKDDDDK Immunomagnetic Beads

Cat. No: EA-IP-001M

Size: 1 mL

Note: Do not centrifuge and use after mixing gently.

Performance metrics

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| Scope of application | Affinity purification and immune (co) precipitation of DYKDDDDK tag fusion protein. DYKDDDDK tag can be located at the N-terminal, C-terminal or middle of the protein, such as N-terminal DYKDDDDK fusion protein (DYKDDDDK–Protein), C-terminal DYKDDDDK fusion protein (Protein–DYKDDDDK) and Met modified N-terminal DYKDDDDK fusion protein (Met–DYKDDDDK–Protein). |
| Antibody properties | Mouse monoclonal antibody, IgG2a subtype. |
| Magnetic beads properties | Agarose coated superparamagnetic beads with an average particle size of 300 nm. |
| Binding capacity | 0.5mL nano magnetic beads covalently coupled with 2mg Anti-DYKDDDDK mouse monoclonal antibody. 0.5mL Anti-DYKDDDDK immunomagnetic beads can purify or precipitate at least 0.6mg DYKDDDDK fusion protein. |
| Repeatability | It can be used repeatedly for more than 5 times. |
| Components | 0.25mL Anti-DYKDDDDK immunomagnetic beads stored in 0.75mL PBS containing preservatives. |

Matters Needing Attention

1. This product is only used for scientific research by professionals, and shall not be used for clinical diagnosis or treatment.
2. For your safety and health, please wear lab clothes and disposable gloves.
3. This product provides affinity magnetic beads in the form of suspension. The content of magnetic beads in the suspension is 25%. Before use, gently re-suspend the magnetic bead suspension, and then use it as required.
4. Do not centrifuge, freeze or dry the magnetic beads do not use sonication for the magnetic beads, and do not allow the acid treatment time of the magnetic beads to exceed 10min.
5. When mixing the magnetic beads, please use the pipette to gently blow, use soft vortex, turn upside down, shaking table mixing and other methods.
6. Related reagents for supporting use shall be prepared by the laboratory itself.

Method of Application

1. **Detection of DYKDDDDK Tagged Protein by Immuno (co) precipitation Method**
 - 1) Gently re-suspend the Anti-DYKDDDDK magnetic beads, mix evenly, take 40 µL magnetic beads suspension (including 10 µL magnetic beads), put it in the centrifuge tube, add 500 µL 1x PBS, fully suspended the suspension and place on the magnetic rack for magnetic separation. After the magnetic rack is left standing for 10 seconds, discard the supernatant. Repeat this washing step twice.
Note: For multiple samples, the magnetic beads can be re-suspended and then divided into several reaction tubes for reaction
 - 2) Add 50-200 µL eukaryotic cell lysate containing target protein, gently re-suspend magnetic beads, and incubate in a shaking table at room temperature for 2h or at 4°C overnight.
 - 3) After standing on the magnetic rack for 10 seconds, transfer the supernatant to a new centrifuge tube for later use (the supernatant can be used to detect whether DYKDDDDK-tag protein remains). Add 500 µL 1xPBS, mix gently, clean the magnetic beads, perform magnetic separation, and discard the supernatant. Repeat twice.
 - 4) Add 20 µL 1xPBS and 5 µL 5x loading buffer, boil the sample for 5min, cool it to room temperature and centrifuge.
 - 5) Take the supernatant for SDS-PAGE test and for subsequent Western Blotting detection.

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2. Purification of DYKDDDDK Tagged Protein by Affinity Purification

1) Magnetic bead pretreatment and sample incubation

- a) Gently re-suspend Anti-DYKDDDDK immunomagnetic beads, mix it evenly, and aspirate 100 µL magnetic beads suspension
- b) (containing about 25 µL magnetic beads) into the centrifuge. Add 900 µL 1xPBS, gently re-suspend the magnetic beads for washing. After the magnetic rack is left standing for 10 seconds, discard the supernatant. Repeat this washing step twice.
- c) Add 50-800 µL eukaryotic cell lysate containing target protein, gently re-suspend magnetic beads, and incubate in a shaking table at room temperature for 2h or at 4°C overnight
- d) After standing on the magnetic rack for 10 seconds, transfer the supernatant to a new centrifuge tube for later use (the supernatant can be used to detect whether there is residual DYKDDDDK tagged protein).
- e) Add 1mL 1xPBS, mix gently, wash the magnetic beads, perform magnetic separation, and discard the supernatant. Repeat four times.
- f) Competitive elution or acid elution can be selected according to protein properties and subsequent experimental requirements.

2) Competitive elution

Competitive elution method has high elution efficiency and strong specificity, does not cause protein denaturation, and is convenient for subsequent analysis and detection of protein.

- a) Add 100 µL or 4 times the volume of magnetic beads 3xDYKDDDDK polypeptide solution, with a concentration of 200µg/mL to the above precipitation, gently re-suspend the magnetic beads, incubate and incubate in a 4°C shaking table for 2h (In order to improve the elution efficiency, the incubation time could be extended or the elution could be repeated).

Note: Adjust the concentration of 3xDYKDDDDK polypeptide solution to 2mg/ml according to the difficulty of protein elution.

- b) Perform magnetic separation and transfer the supernatant containing the target protein to a new centrifuge tube. Repeat this step 1-3 times, if necessary.
- c) Treat and preserve proteins according to the needs of subsequent experiments.

3) Acid elution

Acid elution is a low cost method, has short operation time, and generally does not cause protein denaturation, convenient for subsequent analysis and detection of proteins.

- a) Add pre-cooled acidic eluent 0.5mL or 20 times of the volume of magnetic beads and pH 3.0 to the precipitation. Suspend the magnetic beads and incubate at room temperature for 5 min.

Note: The acid environment will shorten the service life of the immunomagnetic beads. The contact time between the magnetic beads and the acid eluent should be shortened as much as possible. It is recommended that the contact time should not exceed 10min.

- b) After incubation, perform magnetic separation, transfer the supernatant to a new centrifuge tube, and add one tenth of the volume of pH 8.0 neutralizing solution immediately, then mix.
- c) Treat and preserve proteins according to the needs of subsequent experiments.

4) Cleaning and regeneration of magnetic beads

If the immune magnetic beads need to be reused, they must be cleaned and regenerated immediately after elution.

- a) Use acid eluent 10 times the volume of magnetic beads, neutralization solution 10 times the volume of magnetic beads, and 1xPBS of 10 times the volume of magnetic beads for washing.
- b) Rinse with 3 times volume of PBS containing preservatives once more.

- c) Store in PBS containing preservatives of the same volume as magnetic beads, seal at 4°C.

Background

Anti-DYKDDDDK immunomagnetic beads is made by covalently coupling high-quality DYKDDDDK mouse monoclonal antibody and magnetic beads. It has characteristics of high binding capacity of protein, high specificity, fast and convenient operation, stability and repeatability. It can be used for affinity purification and immuno (co) precipitation of DYKDDDDK tagged fusion proteins.

Storage

4°C for 12 months.