Anti- HA（YPYDVPDYA）Immunomagnetic Beads

Cat. No: EA-IP-002M **Size: 1 mL**

Note: *Do not centrifuge and use after mixing gently.*

Performance metrics

Scope of application	Affinity purification and immune (co) precipitation of HA tag fusion protein. HA tag can be located at the N-terminal, C-terminal or middle of the protein, such as N-terminal HA fusion protein (HA‐Protein), C-terminal HA fusion protein (Protein-HA) and Met modified N-terminal HA fusion protein (Met-HA‐Protein).
Antibody properties	Mouse monoclonal antibody, IgG2a subtype.
Magnetic beads properties	Magnetic beads with an average particle size of 3 μm.
Binding capacity	0.5mL nano magnetic beads covalently coupled with 2mg Anti-HA mouse monoclonal antibody. 0.5mL Anti-HA immunomagnetic beads can purify or precipitate at least 0.6mg HA fusion protein
Repeatability	It can be used repeatedly for more than 5 times.
Components	0.25mL Anti-HA 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 HA Tagged Protein by Immuno (co) precipitation Method

1) Gently re-suspend the Anti-HA 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 2hr 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 HA-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.

2. Purification of HA tagged Protein by Affinity Purification

1) Magnetic bead pretreatment and sample incubation

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a) Gently re-suspend Anti-HA immunomagnetic beads, mix it evenly, and aspirate 100 μL magnetic beads suspension (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.

b) 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.

c) 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 HA tagged protein).

d) Add 1mL 1xPBS, mix gently, wash the magnetic beads, perform magnetic separation, and discard the supernatant. Repeat four times.

e) Competitive elution or acid elution can be selected according to protein properties and subsequent experimental requirements.

2) Competitive elution

Competitive elution method, with 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 HA polypeptide solution, with a concentration of 2mg/mL to the above precipitation, gently re-suspend the magnetic beads, incubate and elute 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 HA polypeptide solution to 5mg/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-HA (YPYDVPDYA) immunomagnetic beads is made by covalently coupling high quality HA mouse monoclonal antibody with magnetic beads. It has the 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 HA tagged fusion proteins.

Storage

4°C for 12 months.