Anti-DYKDDDDK Magnetic Beads

Product Packaging:

Product number	Product name	Product form	Package size （V/V, 1/5）
HAK21011-250 ul	Anti-DYKDDDDK Magnetic Beads	20 % suspension	250 uL
HAK21011-1 mL	Anti-DYKDDDDK Magnetic Beads	20 % suspension	1 mL
HAK21011-5 mL	Anti-DYKDDDDK Magnetic Beads	20 % suspension	5 mL
HAK21011-25 mL	Anti-DYKDDDDK Magnetic Beads	20 % suspension	25 mL

Product Description:

The Magnetic Beads are designed for immunoprecipitation and purification of proteins for various research purposes. These beads are covalently conjugated with anti-DYKDDDDK [A2-A4] with a diameter of 30-150 µm, specifically designed to recognize proteins tagged with a FLAG peptide sequence at either the methionine-modified N-terminus or C-terminus. These beads are particularly useful for capturing and purifying FLAG-fused recombinant proteins of mammalian, bacterial or plant origin.

Product performance:

Form: The product is supplied as 20 % slurry in PBS, 0.05 % ProClin™300，pH 7.4

Binding capacity: ≥0.6 mg C-terminal fusion Flag tag protein (~70kDa)/1mL settled magnetic beads

Table 1 Beads binding capacity

The volume (UL) of magnetic beads	Binding capacity (UG).
20	∽12
50	∽30
100	∽60
200	∽120

NOTE: DO NOT use magnetic stirring system which will destroy the magnetic beads structure.

Auxiliary equipment:

magnetic stand, centrifuge tube, 96-well plate.

Storage：

Store at 2-8°C for 2 years. DO NOT freeze or centrifuge Magnetic Beads

Important Licensing Information：

For laboratory or further manufacturing use only.

Instructions for use:

1. Sample preparation

In order to achieve optimal experimental outcomes, careful sample preparation is essential. Follow these guidelines to ensure the quality of your protein lysate:

1.1 Controlling pH and Salt Concentration:

Adjust the pH of the protein lysate to a range of 6 to 8. This pH range enhances binding efficiency during subsequent steps. Maintain a

minimum concentration of 0.15M of NaCl or KCl in the protein lysate. This salt concentration promotes favorable protein interactions.

1.2 Centrifugation for Debris Removal:

Centrifuge the protein lysate at a speed of 10,000 to 20,000 × g for 15 minutes. This centrifugation step effectively removes cellular debris and unwanted particles that could potentially hinder protein binding.For better results, protein lysate can also be filtered through a 0.45 μm or 0.22 μm filter.

2. Immunoprecipitation (IP)

This procedure is recommended for purifying small amounts of Flag fusion protein. It is recommended to use 10-20 μL of magnetic beads suspension per reaction (1x10^6 cells or 500 μL lysate) for IP.

1.Suspend the beads in the bottle thoroughly and transfer the required amount of magnetic beads (see Table 1) into an empty tube.

2. Place the tube into a magnetic stand to collect the beads and discard the supernatant. Be careful not to transfer any beads.

3. Use TBS buffer to wash beads with 10 volumes of the original bead suspension, collect the beads with a magnetic stand, and remove the supernatant. Repeat twice.

4. Add 500 μL of cell lysate sample to the prepared magnetic beads (the volume of the lysate can be adjusted according to the abundance of the target protein in the sample). For the positive control, add 500 uL TBS and ~200ng Flag fusion protein to the pre-washed magnetic beads. For the negative control, add only 500 uL of lysis buffer without protein.

5. Mix by rotation on a rotator for at least 1 hour at room temperature or overnight at 4°C.

6. Collect the beads with a magnetic stand. Remove and discard the supernatant.

7. Use TBS buffer to wash beads with 10 volumes of the original beads suspension. Repeat twice.

8. Elution: According to the requirements of subsequent experiments, one of the following three methods can be selected for elution.

8.1 Elution with acid elution buffer

This is a non-denaturation method, which is fast and efficient. Protein can retained its original biological activity after elution, which was convenient for subsequent analysis and detection.

8.1.1 Preparation of solution: acid eluent (0.1M glycine, 0.2M arginine, pH2.7), neutralizing solution (1M Tris-HCl, pH9.0).

8.1.2 Add 100 uL acid elution to 10ul magnetic beads suspension and mix well, then place on a rotating mixer. Incubate the samples for 5 minutes at room temperature. (Note: Do not leave the beads in this buffer more than 15 minutes.)

8.1.3 Place tube in the magnetic stand to collect the beads. Transfer the supernatants to fresh tubes, and immediately add 10ul neutralizing solution.

8.1.4 Repeat steps 8.1.2-8.1.3 in order to improve elution efficiency, pooling same eluates in tube.

8.1.5 The eluted and neutralized target protein for immediate use, store the eluates at 2-8 °C. Store at –20 °C for long term storage.

Note: 1) Although the acidic elution method is efficient, it may still be lower than the SDS-PAGE sample buffer elution method. 2) Since the difference of the target protein may have a certain influence on the elution efficiency of the acidic elution method, if the elution efficiency is 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 neutralizing solution should also be adjusted to a certain extent.

8.2 Elution with SDS-PAGE Loading Buffer

This is a denaturation method, The protein samples obtained by this method are suitable for SDS-PAGE electrophoresis or Western Blot detection.

8.2.1 Dilute 5X SDS-PAGE loading buffer (Beyotime P0015L) to 1X with PBS.

8.2.2 Add 50 μL 1X SDS sample loading buffer to 10ul magnetic beads suspension and Boil the sample for 10 minutes at 95°C.

8.2.3 Place tube in the magnetic stand to collect the beads. Transfer the supernatants to fresh tube. The sample are ready for loading on SDS-PAGE and immunoblotting.

Notes: Do not re-use magnetic beads after treatment with SDS-PAGE buffer.

8.3 Elution with competitive elution buffer

This is a non-denaturation method, which is fast and efficient. Protein can retained its original biological activity after elution, which was convenient for

subsequent analysis and detection.

8.3.1 Preparation of 3X Flag peptide elution at 300 μg/ml in TBS with 1 %Triton and 0.1 %SDS.

8.3.2 Add 100 uL 3X Flag peptide elution to 10ul magnetic beads suspension and mix well, then place on a rotating mixer. Incubate the samples for 30-60 minutes at room temperature.

8.3.3 Collect the beads with a magnetic stand and then remove the supernatant to a clean tube.The supernatant is the target flag-tagged protein.

8.3.4 Store the eluates at 4 °C for immediate use. Store at –20 °C or –80 °C for long term storage.

The following method was written for affinity purification experiments of small-scale single samples.

For batch purification experiments, it is recommended to use 250 μL magnetic beads suspension per reaction (about 50μL pure magnetic beads). For the use of 96-well plates, it is recommended to use 10 μL pure magnetic beads per well. The amount of magnetic beads can be adjusted according to the abundance of the target protein and the type of magnetic stand.

3. Purification of DYKDDDDK-tagged protein

To purify Flag-tagged proteins, magnetic beads can be used for purification in batches or in 96-well plates. For larger volumes of protein extracts, batch processing is recommended to quickly capture the target protein from a large amount of extracts.

Treatment of Flag fusion protein purification using Anti-DYKDDDDK immunomagnetic beads:

The anti-DYKDDDDK Magnetic Beads are stored in a 20% suspension. Before use, you can choose whether to remove the preservation solution according to the size of the purification system and equilibrate the beads with buffer. Equilibration is performed at room temperature. Only remove the amount of beads required for purification (see Table 1)

1. Suspend the beads in the bottle thoroughly and transfer the required amount of magnetic beads (see Table 1).
2. Transfer the magnetic beads to the appropriate size of the centrifugal tube.Use TBS buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.4) to equilibrate the beads with 10 volumes of the original beads suspension and mix thoroughly. Place the tube into a magnetic stand to collect the beads and discard the supernatant from the tube.
3. Use TBS buffer to wash beads with 10 volumes of the original beads suspension. Repeat twice.
4. Incubate the protein extract (see sample preparation) with magnetic beads (Step 3), Mix by rotation on a rotator for 1 hour at room temperature.
5. After the binding step is completed, collect the beads through magnetic stand. Remove and discard the supernatant.
6. Use TBS buffer to wash beads with 20 volumes of the original beads suspension.Repeat twice.

Note: The washing process can be monitored by measuring the absorbance of the supernatant at 280 nm. Continue washing the beads until the difference in absorbance between the washing solution aspirated from the beads and the wash solution (TBS) blank is< 0.05.

7. Eluted Flag-tagged protein by low pH elution or by competitive elution.

7.1 Elution with Glycine at low PH

Use beads suspension (0.1M glycine, 0.2M arginine, pH2.7) to wash beads with 10 volumes of the original beads suspension, Incubate at room temperature for 5 minutes. Elute the Flag-tagged protein. Add an appropriate amount of neutralizing solution (10μL 1M Tris-HCl, pH 9.0 neutralizing solution per 100ul eluent), and the incubation time should not exceed 15 minutes. Re-equilibrate the beads to neutral pH as soon as possible after elution.

7.2 Elution with 3X Flag peptides competitive elution buffer

Use 3X Flag peptide elution (300 μg/ml) in TBS with 1 %Triton and 0.1 %SDS to wash beads with 5 volumes of the original beads suspension, compete for elution binding Flag fusion protein.

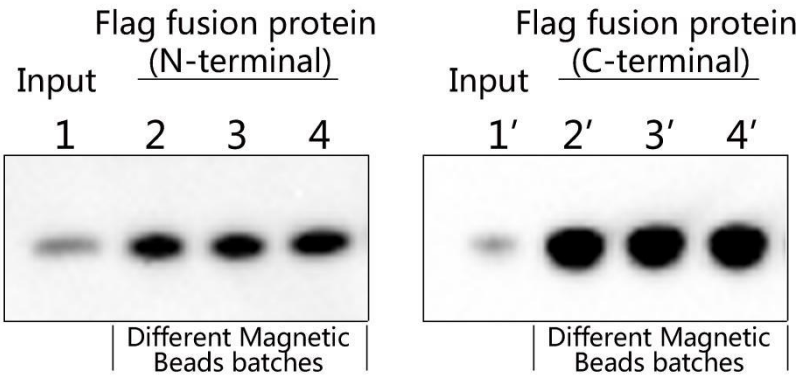
8. Clean magnetic beads

It is recommended to clean the magnetic beads immediately after use by washing the magnetic beads with 3 volumes of eluent (0.1M glycine, 0.2M arginine, pH2.7). The beads should be immediately re-equilibrated in TBS until the effluent is at a neutral pH.

9. Store the beads

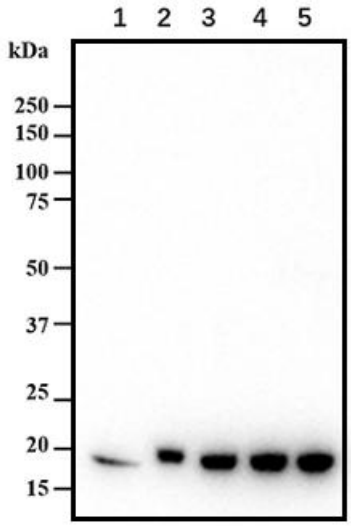
After washing the beads, place the centrifuge tube in an appropriate magnetic stand to collect the beads and remove the buffer. The beads can be supplied as 20 % slurry in PBS, 0.05 % ProClin™300，pH 7.4 . Store the beads at 2-8°C to avoid the beads drying out.

4. Display of results



Immunoprecipitation

Anti-DYKDDDDK [A2-A4] Magnetic Beads: Cat.No. HAK21011: Purification of Two Different FLAG-tagged fusion protein from HeLa transfected with Flag-fusion protein expression vector. 10ul Anti-FLAG [A2-A4] Magnetic Beads was used for IP per lane.
Lane 1, 2, 3, 4: Hela transfected with Flag-fusion protein expression vector containing an N-terminal Flag tag, whole cell lysate
Lane 1', 2', 3', 4': Hela transfected with Flag-fusion protein expression vector containing an C-terminal Flag tag, whole cell lysate



Immunoprecipitation

Lane 1, 2, 3, 4,5: Input, 5μL, 10μL, 15μL, 20 μL Anti-DYKDDDDK [A2-A4] Magnetic Beads suspension were add to the same volume of the Hela lysate transfected with Flag-fusion protein expression vector containing an N-terminal Flag tag, whole cell lysate.