Flag Nanobody Magnetic Beads

（FNB001）

Product Overview

Flag Nanobody Magnetic Beads are prepared by the conjugation of anti-Flag-tag (DYKDDDDK) nanobodies to magnetic agarose beads, enabling specific binding to Flag tag fusion proteins. Unlike conventional antibodies, this product lacks light and heavy chains and has a dissociation constant in the nM-pM range. A 20 μL bead suspension can bind 10~15 μg of Flag tag fusion protein. Featuring high specificity, low background, high affinity, high loading capacity and ease of use, the beads are suitable for experiments such as Immunoprecipitation (IP)/Co-Immunoprecipitation (CoIP), Chromatin Immunoprecipitation (ChIP)/RNA-Binding Protein Immunoprecipitation (RIP), enzyme activity assay and mass spectrometry analysis.

Components

Product Name	Cat. No.	Volume
Flag Nanobody Magnetic Beads	FNB001-01	500 μL
	FNB001-02	1 mL

Storage Conditions

Store at 4℃ for a shelf life of 1 year, or at -20℃ for a shelf life of 2 years. Transport with ice packs.

Notes

1. For your health and safety, wear a lab coat and disposable gloves during operation. This product is for scientific research use only.
2. For long-term storage, store the product at -20℃. Avoid centrifugation, desiccation and repeated freeze-thaw cycles.
3. The storage buffer of this product is PBS containing 20% ethanol.

Experimental Procedures

1. Cell Harvesting：

Approximately 10^6 ~ 10^7 cells expressing Flag-tag fusion protein are used for each immunoprecipitation reaction; the cell number can be adjusted appropriately according to the expression level of the Flag-tag fusion protein. Aspirate the culture medium, add pre-cooled 1×PBS to the culture dish and rinse twice. Harvest adherent cells using a cell scraper or trypsin digestion, transfer the cells to a centrifuge tube, centrifuge at 500 g for 3 minutes and discard the supernatant.

2. Cell Lysis：

1) Add a protease inhibitor to the lysis buffer, and resuspend the cells with 1.0 mL of pre-cooled lysis buffer. For membrane proteins or nuclear/chromatin proteins, RIPA lysis buffer or regular IP lysis buffer combined with sonication can be used for cell lysis.

2) Incubate on ice for 30 minutes, with thorough pipetting every 10 minutes, or perform lysis on a rotator at 4℃.

3) Centrifuge at 20,000 g for 15 minutes at 4℃, transfer the lysate (supernatant) to a new pre-cooled tube and discard the pellet.

Note： The cell lysate can be stored long-term at -80℃ at this stage.

3. **Bead Equilibration (Optional)：** Resuspend the Flag Nanobody Magnetic Beads thoroughly, aliquot 20 μL of the beads into 500 μL of pre-cooled lysis buffer, separate the magnetic beads on a magnetic stand until the supernatant becomes clear, and discard the supernatant (it is recommended to cut the tip of the pipette when aspirating 20 μL of the bead suspension).

4. **Protein Binding：**

1） Add the cell lysate to the equilibrated Flag Nanobody Magnetic Beads (if Step 3 is skipped, directly add 20 μL of the beads to the cell lysate), and incubate on a rotator in a 4℃ refrigerator for 30~120 minutes. If needed, retain 50 μL of the lysate as input for Western Blot analysis.

2） Separate the magnetic beads on a magnetic stand until the supernatant becomes clear, and discard the supernatant.

5. **Bead Washing：** Resuspend the Flag Nanobody Magnetic Beads from the previous step with 1.0 mL of pre-cooled lysis buffer, wash with rotation at 4℃ for 5 minutes, separate the beads on a magnetic stand until the supernatant becomes clear, discard the supernatant and repeat the washing step twice.

6. **Protein Elution (Select the elution method according to downstream applications)：**

1) **Elution with SDS-PAGE Loading Buffer：** Add 20~50 μL of 2×SDS-sample buffer to resuspend the Flag Nanobody Magnetic Beads. Heat at 95℃ for 10 minutes for complete denaturation to dissociate the immunoprecipitation complex from the beads. Then centrifuge at 2,500 g for 3 minutes at 4℃ and collect the supernatant; the collected product can be used for SDS-PAGE and Western Blot analysis.

2) **Acidic Elution：** Add 50 μL of 0.2 M glycine (pH 2.5) to elute the bound protein, incubate for 30 seconds with constant mixing, centrifuge and collect the supernatant into a new centrifuge tube. Immediately add 5 μL of 1.0 M Tris (pH 10.4) to neutralize the acidic glycine solution.

Note： This step can be repeated to improve elution efficiency.

3) **Elution for Flag Fusion Protein:** Add an excess amount of Flag-peptide for elution.