Flag Nanobody Agarose Beads

（FNA001）

Product Overview

Flag Nanobody Agarose Beads are prepared by conjugating anti-Flag-tag (DYKDDDDK) nanobodies to agarose beads, capable of binding Flag-tagged fusion proteins. This product lacks the light and heavy chains of conventional antibodies, with a dissociation constant at the nM-pM level. 20 μL of bead suspension can bind 10–15 μg of Flag-tagged fusion proteins. It features high specificity, low background, high affinity, high binding capacity, and ease of use, suitable for immunoprecipitation (IP)/co-immunoprecipitation (CoIP), chromatin immunoprecipitation (ChIP)/RNA-binding protein immunoprecipitation (RIP), enzyme activity assays, mass spectrometry analysis, and other experiments.

Components

Product Name	Cat. No.	Volume
Flag Nanobody Agarose Beads	FNA001-01	500 μL
	FNA001-02	1 mL

Storage Conditions

Stable for 1 year at 4°C or 2 years at -20°C. Shipped with ice packs.

Precautions

1. For your safety, please wear a lab coat and disposable gloves. This product is for **research use only**.
2. For long-term storage, keep at -20°C. Avoid centrifugation, drying, and repeated freeze-thaw cycles.
3. The storage buffer is PBS containing 20% ethanol and 0.03% NaN_3.

Experimental Protocol

1. Cell Collection:

Use approximately 10^6–10^7 cells expressing Flag-tagged fusion proteins per immunoprecipitation reaction; adjust cell number based on expression level. Aspirate the medium, wash cells twice with pre-chilled 1×PBS, harvest adherent cells using a cell scraper or trypsin digestion, transfer to a centrifuge tube, centrifuge at 500 g for 3 min at 4°C, and discard the supernatant.

2. Cell Lysis：

1) Add protease inhibitors to lysis buffer, resuspend cells in 1.0 mL pre-chilled lysis buffer. For membrane proteins or nuclear/chromatin proteins, use RIPA lysis buffer or standard IP lysis buffer combined with sonication.

2) Incubate on ice for 30 min, mixing thoroughly every 10 min or rotating on a rotary mixer at 4°C.

3) Centrifuge at 20,000 g for 15 min at 4°C, transfer the supernatant (cell lysate) to a new pre-chilled tube, and discard the pellet.

Note： The cell lysate can be stored long-term at -80°C.

3. Bead Equilibration (Optional)： Mix Flag Nanobody Agarose Beads thoroughly, transfer 20 μL to 500 μL pre-chilled lysis buffer, separate beads on a magnetic stand until the supernatant is clear, and discard the supernatant (tip: cut off the end of the pipette tip when aspirating 20 μL bead suspension).

4. Protein Binding：

1） Add cell lysate to the equilibrated Flag Nanobody Agarose Beads (or add 20 μL beads directly to the lysate if Step 3 is skipped). Incubate on a rotary mixer at 4°C for 1–3 h. If needed, save 50 μL lysate as input for Western blot analysis.

2） Centrifuge at 2500 g for 3 min at 4°C and discard the supernatant.

5. Bead Washing： Resuspend beads in 1.0 mL pre-chilled lysis buffer, wash on a rotary mixer at 4°C for 5 min, centrifuge at 2500 g for 3 min at 4°C, discard the supernatant, and repeat washing twice.

6. Protein Elution (Choose method based on downstream application)：

1) SDS-PAGE Sample Buffer Elution：Resuspend beads in 20–50 μL 2×SDS-sample buffer. Denature thoroughly by heating at 95°C for 10 min to release immunoprecipitated complexes from beads, centrifuge at 2500 g for 3 min at 4°C, and collect the supernatant for SDS-PAGE and Western blot analysis.

2) Acidic Elution：Add 50 μL 0.2 M glycine (pH 2.5) to elute bound proteins, incubate for 30 s with constant mixing, centrifuge, transfer supernatant to a new tube, and immediately add 5 μL 1.0 M Tris (pH 10.4) to neutralize acidic glycine.

Note：Repeat this step to improve elution efficiency.

3) Flag Fusion Protein Elution：Add excess Flag-peptide for competitive elution.