HA Nanobody Agarose Beads

（HNA001）

Product Overview

HA Nanobody Agarose Beads are prepared by conjugating anti-HA-tag nanobodies to agarose beads, which can specifically bind HA-tagged fusion proteins. Without the light and heavy chains of conventional antibodies, this product achieves a dissociation constant at the nM–pM level. 20 μL of the bead suspension can bind 10–15 μg of HA-tagged fusion proteins. It features high specificity, clean 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
HA Nanobody Agarose Beads	HNA001-01	500 μL
	HNA001-02	1 mL

Storage Conditions

Stable for 1 year at 4°C, and 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, store 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：

Approximately 10^6–10^7 cells expressing HA-tagged fusion proteins are used per immunoprecipitation reaction; adjust the cell number according to the expression level of the HA-tagged fusion protein. Aspirate the medium, wash the dish twice with pre-chilled 1×PBS, harvest adherent cells using a cell scraper or trypsinization, transfer cells to a centrifuge tube, centrifuge at 500 g for 3 min, and discard the supernatant.

2. Cell Lysis：

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

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

3) Centrifuge at 20,000 g for 15 min at 4°C, transfer the lysate (supernatant) 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 HA Nanobody Agarose Beads thoroughly, transfer 20 μL of the product to 500 μL pre-chilled lysis buffer, separate the beads on a magnetic stand until the supernatant is clear, and discard the supernatant (tip: cut off the tip of the pipette tip when aspirating 20 μL of the bead suspension).

4. Protein Binding：

1） Add the cell lysate to the equilibrated HA Nanobody Agarose Beads (if Step 3 is omitted, add 20 μL of the product directly to the cell lysate). Incubate on a rotary mixer at 4°C for 1–3 h. If needed, save 50 μL of the 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 the HA Nanobody Agarose 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 the beads in 20–50 μL 2×SDS-sample buffer. Heat at 95°C for 10 min for full denaturation to release the immunoprecipitated complexes from the beads, then centrifuge at 2500 g for 3 min at 4°C 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 proteins, incubate for 30 s with constant mixing, centrifuge, transfer the supernatant to a new centrifuge tube, and immediately add 5 μL of 1.0 M Tris (pH 10.4) to neutralize the acidic glycine.

Note：Repeat this step to improve elution efficiency.

3) HA-tagged Protein Elution：Add excess HA-peptide for elution.