



PRODUCT SPECIFICATION

Recombinant anti-human Vinculin nanobody 85.

Catalogue number: sdAb-Vinculin-Nb85

Background :

Vinculin is a cytoskeletal protein of 1066 amino acids (~117 kDa). It interacts with several other cytoskeletal proteins including talin and α -actinin, but also F-actin. It links the cytoskeleton to the membrane of cells and to integrins. Vinculin is a prominent component (and regulator) of focal adhesions and adherens junctions, and is involved in cell spreading (lamellipodium formation) and adhesion.

Talin, α -actinin

VASP, Mena, Arp2/3

PIP₂, F-actin, paxillin

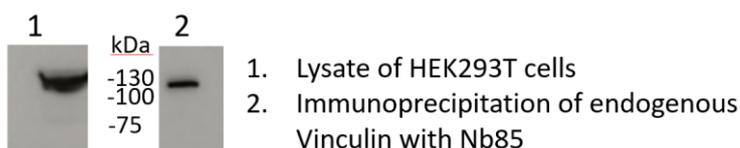


Schematic domain structure of vinculin, highlighting some of its main interaction partners.

Applications: PD, IP, ELISA. This product is for R&D use only, not for drug, diagnostic, therapeutic, household, or other uses. Not suitable for WB.

Nanobody functionality: Immunoprecipitation of endogenous Vinculin from HEK293T cells with recombinant Vinculin Nb 85.

Procedure: 1 mg protein extract from HEK cells (lyzed in 20 mM Tris/HCl pH 7.5, 1 % Triton X-100, inhibitor cocktail and PMSF) was incubated with 1 μ g HA-tagged Vinculin nanobody 85 for 1 hour at 4°C. Next, this mixture was added to 10 μ l anti-HA antibody coupled to settled sepharose beads, again for 1 hr at 4°C. Following 4 washes with 1 ml lysis buffer, Laemmli sample buffer was added to the beads and boiled for 2 minutes. The supernatant was size fractionated by SDS-PAGE (10%) and then proteins were transferred to nitrocellulose by conventional blotting. The blot was blocked with 5% milk powder in Tris buffered saline. Primary antibody was rabbit monoclonal anti Vinculin Ab (1/10000 dilution). A HRP-coupled antibody was used as secondary. Finally, the blot was exposed to hyperfilm for 10 seconds.



Source and properties:

Vinculin nanobody 85 was raised by immunizing a llama with a recombinant protein fragment comprising the C-terminal half of vinculin. Nb 85 binds to the Vinculin fragment with a K_d of 3.8×10^{-8} M ($\pm 4.90 \times 10^{-11}$ M).

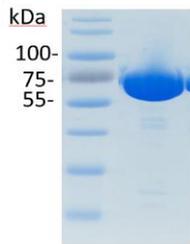


Figure: purified Vinculin fragment used for immunization. SDS-PAGE (10% gel) followed by Coomassie staining. Protein standards are in kDa.

- Availability: Vinculin Nanobody 85 comes with a COOH-terminal HA epitope tag. Available in 100 µg, 500 µg, 1000 µg quantities. For bulk amounts, please inquire.
- Expression host: VHH single domain antibody purified from *E. coli*.
- Cross reactivity: Reactivity of this nanobody with vinculin from other species has not been tested.
- Storage buffer: 20 mM Tris-HCl pH 8.0, 150 mM NaCl, 1mM DTT, 60 % glycerol. Store at -20°C. The sample will not freeze. Maintain sample in cold environment during transport to increase longevity.
- Stability: Store at -20°C upon arrival. For long term storage, aliquot and store at -80°C. Avoid repeated freeze/thaw cycles.

Product citations:

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