Nanobody toolbox for your research

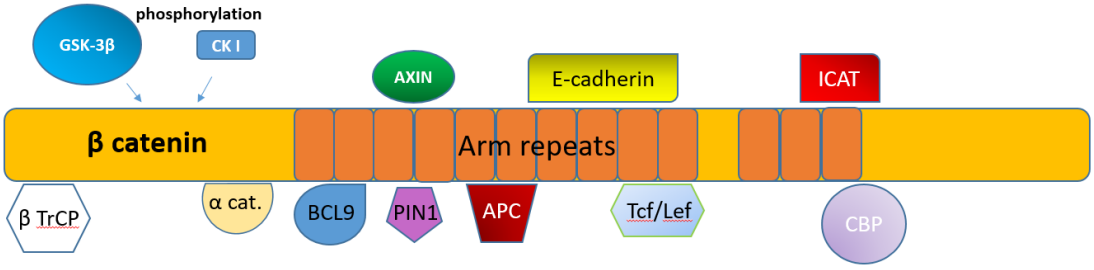
PRODUCT SPECIFICATION

Recombinant anti-human β-catenin nanobody 77.

Catalogue number: sdAb-CAT-Nb77

Background

β-catenin, encoded by the *CTNNB1* gene, is involved in cell-cell adhesion and represents an important component of the Wnt signaling pathway. Pathological mutations in beta-catenin are associated with different types of cancer, but also with heart disease.

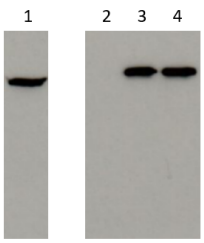


Model depicting β-catenin and (some of) its interaction partners

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

Nanobody functionality:

Immunoprecipitation of endogenous β-catenin from HEK 293T cell extracts with beta catenin nanobody.



1. HEK 293T cell lysate 80 µg
2. Anti HA tag-antibody + HEK lysate
3. Immunoprecipitation β-catenin Nb 77
4. Immunoprecipitation β-catenin Nb 86

Procedure: 1 mg protein extract from HEK 293T cells (lyzed in 20 mMTris/HCl pH 7.5, 1 % Triton X-100, inhibitor cocktail and PMSF) was incubated with 1 µg HA-tagged beta catenin nanobody 77 for 1 hour at 4°C. Next, this mixture was added to 10 µl 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 (8%) 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 against beta-catenin at 1:5000 dilution. A HRP-coupled antibody was used as secondary. Finally, the blot was exposed for 10 seconds to hyperfilm.

Source and properties

β-catenin nanobody 77 was raised by immunizing a llama with the C-terminal half of recombinant human β-catenin. Nanobody 77 binds to the catenin fragment with a K_d of **4,88 × 10^{-9} M** ± 3.93 × 10^{-11} M. It may bind an epitope in catenin different form Nb86.

Availability: Beta-catenin Nanobody 77 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 catenin from species other than human 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.

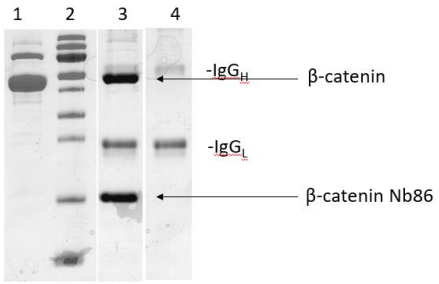
Stability: Store at -20°C upon arrival. For long term storage, aliquot and store at -80°C. Avoid repeated freeze/thaw cycles.

Product citations: /

Pull down experiment on the antigen:

Partially purified beta-catenin (Figure below, lane 1) was incubated with HA-tagged Nb77 and the complex retrieved on anti-HA agarose. Following boiling in Laemmli sample buffer, the immunoprecipiate was analyzed by SDS-PAGE (15%) and stained with Coomassie brilliant blue. IgG_L and IgG_H leaked slightly from anti-HA agarose beads.

Note that nanobody and beta-catenin stain virtually equally intense on the gel, indicative of a strong binder. This is confirmed in the K*d* (5nM).



Legend:

1. Recombinant β-catenin, partially purified
2. Protein marker
3. Pull down with β-catenin Nb 77
4. Negative control: anti-HA agarose + β-catenin

The catenin fragment used for immunization is shown below:

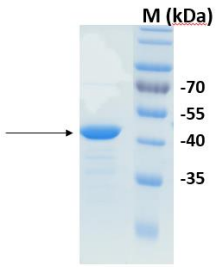


Figure: purified catenin fragment covering the C-terminal half of the protein, used for immunization. SDS-PAGE (10% gel) followed by Coomassie staining. M = protein standards (kDa).