Nanobody toolbox for your research

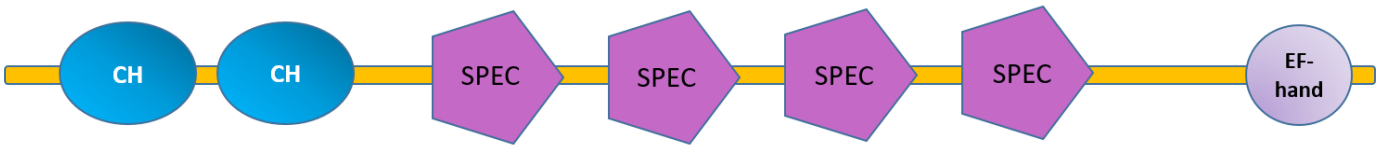
PRODUCT SPECIFICATION

Recombinant anti-human alfa-actinin 4 nanobody 64.

Catalogue number: sdAb-ACT4-Nb64.

Background

Alfa-actinin4 is a cytoskeletal protein and actin cross-linker encoded by the *ACTN4* gene, and is a member of the spectrin superfamily of cytoskeletal proteins. Together with isoform 1, actinin-4 is found in nearly every non-muscle cell but also in cancer cells. The N-terminal pair of Calponin-homology domains constitute an actin binding site whereas the spectrin repeats likely form a rod-like domain. It forms homodimers and has been observed to shuttle between nucleus and cytoplasm under certain conditions. Alfa-actinin function is not restricted to cell motility and cytoskeletal organization, but extends to modulation of gene expression, apoptosis and endocytosis. Actinin-4 deficient mice develop proteinurea and glomerular disease.

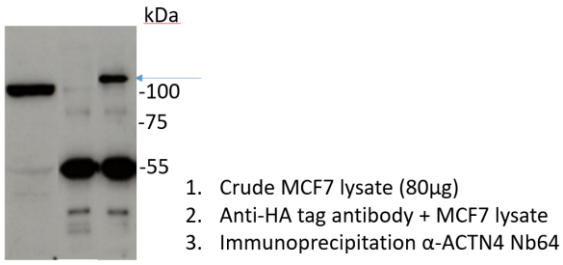


Model depicting alfa-actinin4 with its 2 NH_2–terminal calponin homology domains, 4 central spectrin repeats, and COOH-terminal EF-hand.

Applications: PD, IP, ELISA. Other applications have not yet been tested. This product is for R&D use only, not for drug, diagnostic, therapeutic, household, or other uses. Not suitable for Western blot.

Nanobody functionality:

Immunoprecipitation of endogenous α-actinin 4 from MCF7 cell extracts with actinin 4 nanobody.



Procedure: 1 mg protein extract from MCF7 cells (lyzed in 20 mMTris/HCl pH 7.5, 1 % Triton X-100, inhibitor cocktail and PMSF) was incubated with 1 µg HA-tagged actinin 4 nanobody 64 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 mouse monoclonal against actinin 4 at 1:1000 dilution. A HRP-coupled antibody was used as secondary. Finally, the blot was exposed hyperfilm.

Source and properties

Alfa-actinin 4 nanobody 64 was raised by immunizing a llama with a protein fragment encompassing actinin 4 amino acids 404-765. Nanobody 64 binds to the actinin fragment with a K_d of **1.2×10^{-8} M**.

The actinin4 fragment used for immunization is shown below:

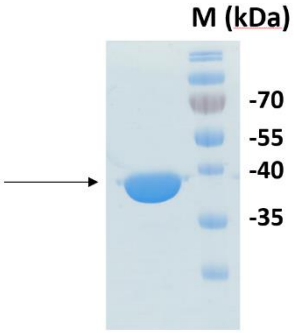


Figure: purified actinin 4 fragment used for immunization. SDS-PAGE (10% gel) followed by Coomassie staining. M = protein standards (kDa).

Availability: Alfa-actinin 4 nanobody 64 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 alfa-actinin 4 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:

/

Please enquire about other actinin 4 nanobodies at info@gulliverbiomed.com