Nanobody toolbox for your research

PRODUCT SPECIFICATION

Recombinant anti-human VASP nanobody 80.

Catalogue number: sdAb-VASP-Nb80.

Background

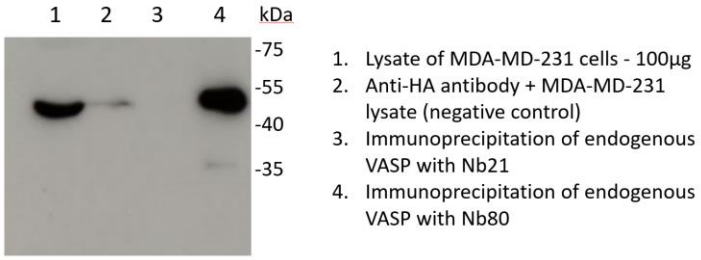
Vasodilator-stimulated phosphoprotein (VASP) is a protein of 380 amino acids and belongs to the Ena-VASP family of modular actin associated proteins. They are built up of an N-terminal EVH1 domain, a proline-rich region in the middle and a C-terminal EVH2 domain. The first domain indirectly mediates targeting to focal adhesions while the EVH2 domain mediates VASP tetramerization, and binds to actin. VASP is phosphorylated by protein kinases A and G. Its phosphorylation is used to monitor antiplatelet drugs as it is involved in the regulation of platelet aggregation.

VASP interacts with a number of other (cytoskeletal) proteins, including profilins, zyxin, and the *Listeria* protein ActA. It can be found at the leading edge of lamellipodia and in filopodia. This family of proteins contributes to cell migration and axon guidance.

Model depicting human VASP with its N-terminal EVH1 domain, central PRR domain and C-terminal EVH2 region.

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 VASP from MDA-MB-231 breast cancer cells with VASP Nb 80.



PS. VASP Nanobody 21 in lane 3 was identified as a positive binder during nanobody isolation but did not succeed in immunoprecipitating endogenous VASP, in contrast to Nb80.

Procedure: 1 mg protein extract from MDA-MB-231 cells (lyzed in 20 mMTris/HCl pH 7.5, 1 % Triton X-100, inhibitor cocktail and PMSF) was incubated with 1 µg HA-tagged VASP nanobody 80 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 (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 polyclonal anti VASP Ab (1/1000 dilution). A HRP-coupled antibody was used as secondary. Finally, the blot was exposed to hyperfilm for 10 seconds.

Source and properties

VASP nanobody 80 was raised by immunizing a llama with a protein fragment encompassing human VASP amino acids 1-343. Nb 80 binds to the VASP fragment with a K_d of **5.07×10^{-9} M** ($\pm$ 4.78×10^{-11}M).

The VASP fragment used for immunization is shown below:

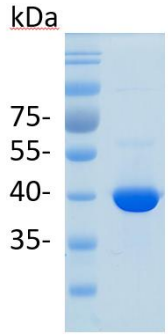


Figure: purified VASP fragment used for immunization. SDS-PAGE (10% gel) followed by Coomassie staining. Protein standards are in kDa.

Availability: VASP nanobody 80 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: Species other than human have not yet 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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