Nanobody toolbox for your research

PRODUCT SPECIFICATION

Recombinant anti-human c-Src nanobody 76 & 35.

Catalogue number: sdAb-Src-Nb35 or sdAb-Src-Nb76

Background

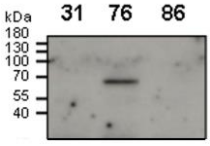
The kinase Src is a non receptor tyrosine kinase of a kinase family bearing the same name. As a tyrosine kinase it phosphorylates numerous substrates on tyrosine residues, thus controlling their activity or localization. Perturbation of Src contributes to cell transformation. Src is activated by immune response receptors, GPCRs, cytokine receptors, among others. Src activity contributes to cancer cell invasion and metastasis, cell cycle progression, apoptosis, cytoskeletal organization (i.e. cortactin phosphorylation) and many other cellular phenomena. We refer to the many excellent reviews that have been written on this intreaguing and multi-faceted protein kinase in the scientific literature.

Source: Wikipedia, Uniprot https://www.uniprot.org/uniprot/P12931

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

Nanobody functionality:

Immunoprecipitation of endogenous c-Src from HEK293T cell extracts with Src nanobody 76.



Procedure: 1 mg protein extract from HEK293T cells (lyzed in 20 mMTris/HCl pH 7.5, 1 % Triton X-100, inhibitor cocktail and PMSF) was incubated with ~1 µg HA-tagged Src nanobody 76 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 (15%) 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 polyclonal anti Src Ab 1/1000. A HRP-coupled antibody was used as secondary (1/2000). Finally, the blot was exposed to hyperfilm.

'76' in the western blot above refers to Src nanobody 76.

The numbers 31 and 86 refer to immunoprecipitation with Src nanobodies 31 and 86. These were also identified as high affinity binders following phage panning with the recombinant antigen as expressed and purified from bacteria, but proved not to be suitable for binding the endogenous protein.

Source and properties:

Src nanobodies 35/76 were raised by immunizing a llama with a recombinant Src fragment comprising amino acids 1-252. Nb 76 binds with an affinity of 1.63×10^{-8} M (**16 nM**), while Nb 35 binds with an affinity of 5.09×10^{-9} M (**5 nM**). Both are able to pull down endogenous c-Src from HEK293T cells.

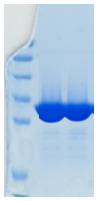


Figure: purified human Src fragment 1-252 used for immunization. The gel was stained with Coomassie brilliant blue.

Src nanobodies 35 and 76 have different CDR3 amino acid sequences, suggesting they may bind to different epitopes in the antigen.

Availability: Src Nanobody 35 or 76 come with a COOH-terminal HA or Myc 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 Src 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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