Nanobody toolbox for your research

PRODUCT SPECIFICATION

Recombinant anti-mouse Focal adhesion kinase (FAK) nanobody 54.

Catalogue number: sdAb-FAK-FAT-Nb54.

Background

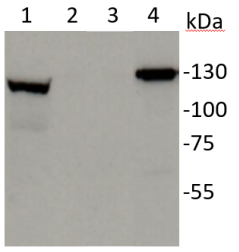
Focal adhesion kinase is a cytosolic (non-receptor) tyrosine kinase of 125 kDa, concentrated in focal adhesions. The FERM (4.1 protein, Ezrin, Radixin, Moesin) and kinase domains form an autoinhibitory association. The FAT domain (focal adhesion targeting domain) directs the kinase to focal adhesions. FAK is found in most cells. FAK promotes cell migration by activating a signaling pathway that induces turn-over of cell contacts with the underlying matrix. During apoptosis, FAK is cleaved by caspase 3 and contributes to loss of focal contacts, cell rounding and formation of blebs. At the organismal level, FAK is required for normal early embryonic development, placenta and heart development. FAK regulates numerous signaling pathways (integring signal transduction, GPRCs, LDL, netrin receptors. Substrates:Src kinase, BMX, PIK3R1, PAX, STAT1,…

Model depicting human Focal Adhesion Kinase with its N-terminal FERM domain, central kinase domain and C-terminal FAT domain.

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. Reacts with human and mouse FAK.

Nanobody functionality:

Immunoprecipitation of endogenous FAK from HEK2993T with FAK FAT nanobody 54.

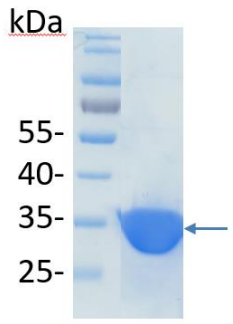


1. Crude lysate HEK cells 80µg
2. Anti-HA antibody + HEK lysate (negative control)
3. Protein marker
4. Immunoprecipitation endogenous FAK with Nb54

Procedure: 1 mg protein extract from HEK283T cells (lyzed in 20 mMTris/HCl pH 7.5, 1 % Triton X-100, inhibitor cocktail and PMSF) was incubated with 1 µg HA-tagged FAK nanobody 54 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 anti FAK 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

FAK FAT nanobody 54 was raised by immunizing a llama with a protein fragment encompassing mouse FAK amino acids 750-1053. Nb 54 binds to the FAT fragment with a K_d of **1.65×10^{-9} M** ($\pm 1.89 \times 10^{-11}$M).



The FAK fragment used for immunization is shown below:

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

Availability: FAK FAT nanobody 54 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 mouse and human FAK has been established. Other species 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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Please enquire about other FAK FAT nanobodies at info@gulliverbiomed.com