



## PRODUCT SPECIFICATION

Recombinant anti-human Tks5 nanobody 10 & 77.

Catalogue number: sdAb-Tks5-Nb10 or sdAb-Tks5-Nb77

### Background

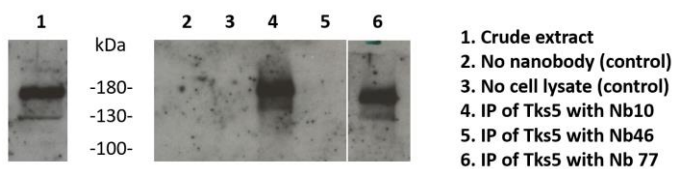
The adaptor protein Tks5 (aka FISH, SH3PXD2A) is an 1105 amino acid protein built up of an N-terminal PX domain (phosphoinositide binding), followed by 5 SH3 domains/interaction platforms. This protein is a component of cancer cell invadopodia and immune cell podosomes and is involved in the degradation by some cancer cells of the extracellular matrix. Tks4 is a close relative.



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

### Nanobody functionality:

Immunoprecipitation of endogenous Tks5 from SCC-61 cancer cell extracts with Tks5 nanobody 10 or Tks5 nanobody 77.



Procedure: 1 mg protein extract from SCC-61 head and neck squamous cell carcinoma cells (lyzed in 20 mM Tris/HCl pH 7.5, 1 % Triton X-100, inhibitor cocktail and PMSF) was incubated with 2 µg HA-tagged Tks5 nanobody 10 or 77 for 1.5 hour at 4°C. Next, this mixture was added to 20 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 Tks5 Ab 1/1000 dilution. A HRP-coupled antibody was used as secondary (1/2000). Finally, the blot was exposed to hyperfilm for 1 minute.

Lane 5 represents an immunoprecipitation with Tks5 nanobody 46. This nanobody was also identified as a high affinity binder (with the recombinant antigen as expressed and purified from bacteria) but apparently is not suitable for binding the endogenous protein.

Source and properties:

Tks5 nanobodies 10/77 were raised by immunizing a llama with a Tks5 fragment comprising the two most C-terminal SH3 domains. Nb 10 binds with an affinity of  $2.58 \times 10^{-9}$  M (**2.58 nM**,  $\pm 2.93 \times 10^{-11}$  M). Tks5 Nb 77 binds with an affinity of  $2.50 \times 10^{-9}$  M (**2.50 nM**,  $\pm 2.46 \times 10^{-11}$  M). Both are able to pull down endogenous Tks5 from SCC-61 cancer cells.

They have different CDR3 sequences, suggesting they may bind to different epitopes in the antigen.

Availability: Tks5 Nanobody 10 or 77 come with a COOH-terminal HA or Myc epitope tag. Available in 100  $\mu$ g, 500  $\mu$ g, 1000  $\mu$ g quantities. For bulk amounts, please inquire.

Expression host: VHH single domain antibody purified from *E. coli*.

Cross reactivity: Reactivity of this nanobody with Tks5 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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