

## PRODUCT SPECIFICATION

Recombinant anti-human Annexin A5 (V) nanobody 36.

Catalogue number: sdAb-ANNEX-Nb36.

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Recombinant anti-human Annexin A5 (V) nanobody 59.

Catalogue number: sdAb-ANNEX-Nb59.

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### Background

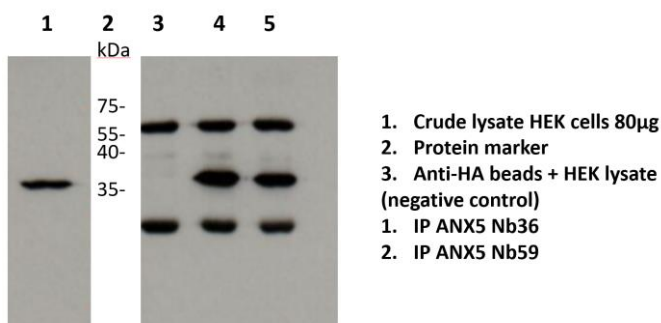
Annexins constitute a protein family and Annexin A5 consists of 4 'annexin repeats'. This polypeptide of 36 kDa has been used often as an indicator of apoptosis in flow cytometry experiments. It binds to the negatively charged phospholipid phosphatidylserine, a typical phospholipid encountered in many cell membranes. Phosphatidylserine is a marker of apoptosis when it is exposed on the plasma membrane outer leaflet. In antiphospholipid syndrome, anti-annexin A5 antibodies are found in patients. Annexin A5 has been shown to interact with integrin beta 5.

Sources: Annexins in Glaucoma, doi: 10.3390/ijms19041218. Wikipedia.

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.

### Nanobody functionality:

#### Immunoprecipitation of endogenous annexin A5 from MCF7 cell extracts with Annexin A5 nanobodies 36 and 59.



Procedure: 1 mg protein extract from HEK293T cells (lyzed in 20 mM Tris/HCl pH 7.5, 1 % Triton X-100, inhibitor cocktail and PMSF) was incubated with 1 µg HA-tagged annexin A5 nanobody 36 or 59 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 a mouse monoclonal anti-ANX5 Ab 1/1000 dilution. A HRP-coupled antibody was used as secondary. Finally, the blot was exposed to hyperfilm (7 minutes).

## Source and properties

Annexin nanobody 36/59 was raised by immunizing a llama with the full length recombinant protein.

Nanobody 36 binds to annexin A5 with a  $K_d$  of  $2.49 \times 10^{-8} \text{ M}$  (~25 nM)  $\pm 1.54 \times 10^{-10} \text{ M}$ .

Nanobody 59 binds to annexin A5 with a  $K_d$  of  $2.45 \times 10^{-8} \text{ M}$  (~25 nM)  $\pm 1.53 \times 10^{-10} \text{ M}$ .

The annexin antigen used for immunization is shown below:

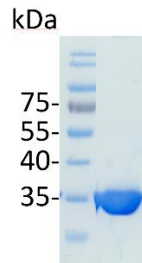


Figure: purified human annexin A5 used for immunization. SDS-PAGE (15% gel) followed by Coomassie staining.

Availability: Annexin A5 nanobodies 36 and 59 come 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 annexin A5 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:

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