

PRODUCT SPECIFICATION

Recombinant anti-human Bcl-2 nanobody 88/92/93.

Catalogue number: sdAb-Bcl2-Nb88, 92 or 93.



Background

Contact us if you want to use the Bcl-2 nanobody cDNA through info@gulliverbiomed.com. We do not regularly distribute cDNAs encoding nanobodies except in collaborative studies.

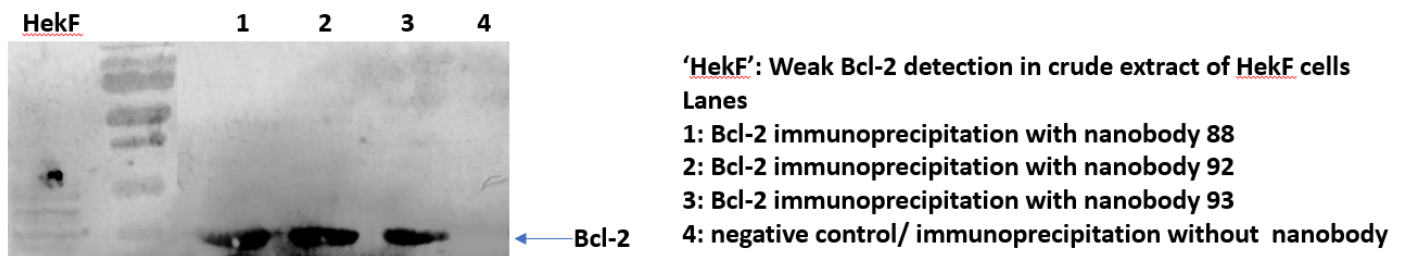
Bcl-2 stands for B-cell lymphoma 2. Although Bcl-2 is known to regulate mitochondrial dynamics, fusion and fission, the protein is best known for its role in (countering) apoptosis. It can be found at the outer mitochondrial membrane where it inhibits pro-apoptotic proteins such as Bak and Bax. In some cancers, Bcl-2 is overexpressed which protects cancer cells against apoptosis. However, dysregulation of other genes, such as the transcription factor Myc, is required to promote malignancies.

Sources: Wikipedia, Zhang et al. <https://doi.org/10.1016/j.bbcan.2021.188569>

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 Bcl-2 protein from HekF cell extracts with Bcl-2 nanobody 88, 92 or 93.



Procedure: 1 mg protein extract from HekF 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 Bcl-2 nanobody 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 for detection was a monoclonal against Bcl-2 at 1:1000 dilution. A HRP-coupled antibody was used as secondary.

Source and properties

Bcl-2 nanobodies 88, 92 and 93 were raised by immunizing a llama with the full length human protein. Epitope mapping has not been performed. Possibly different nanobodies interact with different Bcl-2 epitopes but this can only be established experimentally. The three Nanobodies have different CDR3 sequences which may reflect interaction with different regions in the target protein.

Affinity (determined by Octet):

Bcl-2 Nanobody 88 binds to Bcl-2 with a K_d of $2.31 \times 10^{-9} \text{ M}$ ($\pm 1,45\text{E} \times 10^{-11} \text{ M}$).

Bcl-2 Nanobody 92 binds to Bcl-2 with a K_d of $8.62 \times 10^{-9} \text{ M}$ ($\pm 1,34\text{E} \times 10^{-10} \text{ M}$).

Bcl-2 Nanobody 93 binds to Bcl-2 with a K_d of $7.48 \times 10^{-9} \text{ M}$ ($\pm 7,07\text{E} \times 10^{-11} \text{ M}$).

Availability: Bcl-2 nanobodies 88, 92 or 93 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 Bcl-2 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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Please enquire about other Bcl-2 nanobodies at info@gulliverbiomed.com