

PRODUCT SPECIFICATION

Recombinant anti-green fluorescent protein nanobody 94.

Catalogue number: sdAb-GFP-Nb94



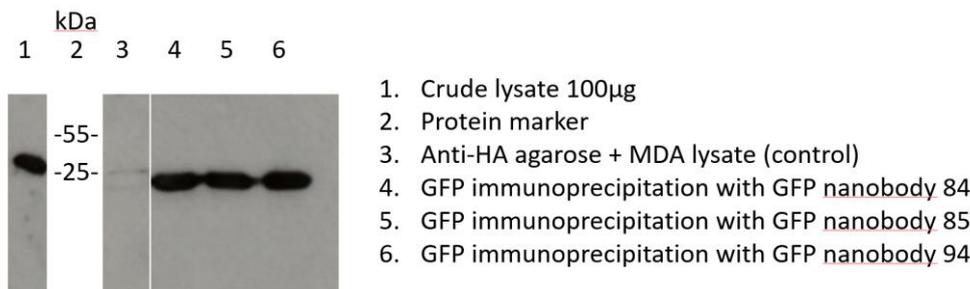
Background

The green fluorescent protein from *Aequorea Victoria* does not require extensive introduction. It has been used in probably thousands of studies in biomedicine and biophysics, and it is a truly remarkable intrinsically fluorescent protein that sparked discovery and use of many other fluorescent proteins. GFP has been, and still is, an important research tool in many studies and for this reason we present here our GFP nanobodies. Please enquire for other GFP which are available at Gulliver Biomed.

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

Nanobody functionality:

Immunoprecipitation of GFP from MDA-MB-231 human breast cancer cells which were transduced with a lentiviral construct encoding green fluorescent protein (a so-called stable cell line).



Procedure: 1 mg protein extract from MDA-MB-231 cells (lyzed in 20 mM Tris/HCl pH 7.5, 1 % Triton X-100, inhibitor cocktail and PMSF) was incubated with 1 µg of the respective HA-tagged GFP nanobodies 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 an anti rabbit GFP antibody 1/100 dilution. A HRP-coupled antibody was used as secondary. Finally, the blot was exposed hyperfilm.

Source and properties

GFP nanobodies were raised by immunizing a llama with recombinant GFP.

Nanobody 84 binds to GFP with a K_d of 6×10^{-8} M (60 nM), $\pm 2,55 \times 10^{-9}$ M.

Nanobody 85 binds to GFP with a K_d of 8×10^{-9} M (8 nM), $\pm 1,05 \times 10^{-10}$ M.

Nanobody 94 binds to GFP with a K_d of 7×10^{-9} M (60 nM), $\pm 6,24 \times 10^{-11}$ M.

Availability: GFP nanobodies 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*.

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 GFP nanobodies at info@gulliverbiomed.com