



tdTomato Nanoselector Agarose

Summary

Catalog No	026-101-002
Ligand	Anti-tdTomato single domain antibody fragment (VHH, Nanobody)
Bead size	~ 40 μ m
Reactivity	tdTomato
Binding capacity	High binding capacity, 10 μ L slurry bind about 20 μ g of recombinant tdTomato
Storage	Shipped at ambient temperature. Upon receipt store at 4°C. Stable for 1 year. Do not freeze
Storage buffer	50 % slurry in PBS containing 20 % Ethanol

Description

tdTomato Nanoselector Agarose has been specifically designed to bind tdTomato-fusion proteins. tdTomato Nanoselector Agarose is based on small high-affinity recombinant single domain antibody covalently coupled to the surface of Agarose beads. tdTomato Nanoselector Agarose is an ideal tool to isolate or purify tdTomato-fusion proteins fast and efficiently.

Background

The tandem dimer Tomato (tdTomato) is a genetically improved version of *Discosoma* sp. fluorescent protein (dsRed). tdTomato is an extremely bright red fluorescent protein, being one of the brightest red-shifted fluorescent proteins available and six times brighter than eGFP. One of the main advantages of fluorescent proteins is that they do not require substrates or reagents for detection, however most of them do need oxygen for the formation of their chromophores. Besides, fluorescent proteins can be measured without lysing cells, whereas this is difficult for luciferases. One of the biggest disadvantages of fluorescent reporters is that they need to overcome cellular autofluorescence. However, in general cellular autofluorescence is lower for the emission wavelength of the red channel, favoring a red fluorescent protein such as tdTomato.

For biochemical analysis including mass spectrometry and enzyme activity measurements these tdTomato-fusion proteins and their interacting factors need to be isolated fast and efficiently by immunoprecipitation using the tdTomato Nanoselector Agarose. Due to the single-chain nature of sdAbs and their stable and covalent attachment, no leakage of light and heavy chains is observed during elution with SDS sample buffer. tdTomato Selector is compatible not only with physiological buffers but also with high stringency buffers which provides great freedom to adjust the binding and washing conditions to the experimental needs.

Application notes

Immunoprecipitation (IP)/Co-IP
 Mass spectrometry (MS)
 Enzyme activity measurements

Benefits

- Effective pulldown of tdTomato-fusion proteins for consistent results
- No heavy & light antibody chains, short incubation (5-30 min)
- Extraordinary binding, even under harsh conditions
- Very high affinity to bind even low abundant proteins

Immunoprecipitation protocol

Mammalian cell lysis

Note: Harvesting of cells and cell lysis should be performed with ice-cold buffers. We strongly recommend to add protease inhibitors to the Lysis buffer to prevent degradation of your target protein and its binding partners. For one immunoprecipitation reaction, we recommend using $\sim 10^6$ - 10^7 cells.

1. Choice of lysis buffer:

* For cytoplasmic proteins, resuspend the cell pellet in 200 μ L ice-cold Lysis buffer by pipetting up and down. Supplement Lysis buffer with protease

* For nuclear/chromatin proteins, resuspend cell pellet in 200 μ L ice-cold RIPA buffer supplemented with DNaseI (f.c. 75-150 Kunitz U/mL), MgCl₂ (f.c. 2.5 mM), protease inhibitor cocktail and PMSF (f.c. 1 mM) (not included)

2. Place the tube on ice for 30 min and extensively pipette the suspension every 10 min.

3. Centrifuge cell lysate at 17,000x g for 10 min at +4°C. Transfer cleared lysate (supernatant) to a pre cooled tube and add 300 μ L Dilution buffer supplemented with 1 mM PMSF and protease inhibitor cocktail (not included). If required, save 50 μ L of diluted lysate for further analysis (input fraction).

This product is for research use only and is not approved for use in humans or in clinical

Beads equilibration

1. Resuspend the beads by gently pipetting up and down or by inverting the tube. Do not vortex the beads!
2. Transfer 25 μ L of bead slurry into a 1.5 mL reaction tube.
3. Add 500 μ L ice-cold Dilution buffer.
4. Separate the beads with a magnet until the supernatant is clear.
5. Discard the supernatant.

Protein binding

1. Add diluted lysate to the equilibrated beads.
2. Rotate end-over-end for 1 hour at +4°C.

Washing

1. Separate the beads with a magnet until the supernatant is clear.
2. If required, save 50 μ L of supernatant for further analysis(-flow-through/non-bound fraction).
3. Discard remaining supernatant.
4. Resuspend beads in 500 μ L Wash buffer.
5. Separate the beads with a magnet until the supernatant is clear. Discard the remaining supernatant.
6. Repeat this step at least twice.
7. During the last washing step, transfer the beads to a new tube.

Optional: To increase stringency of the Wash buffer, test various salt concentrations e.g. 150 mM - 500 mM, and/or add a non-ionic detergent e.g. Triton™ X-100.

Elution with 2x SDS-sample buffer

1. Remove the remaining supernatant.
2. Resuspend beads in 80 μ L 2x SDS-sample buffer.
3. Boil beads for 5 min at +95°C to dissociate immunocomplexes from beads.
4. Separate the beads with a magnet.
5. Analyze the supernatant in SDS-PAGE.

Elution with Glycine-elution buffer

1. Remove the remaining supernatant.
2. Add 50–100 μ L Glycine-elution buffer and constantly pipette up and down for 30 - 60 sec at +4°C.
3. Separate the beads with a magnet until the supernatant is clear..
4. Transfer the supernatant to a new tube.
5. Immediately neutralize the eluate fraction with Neutralization buffer.
6. Repeat this step at least once to increase elution efficiency .

Suggested buffer compositions

Buffer	Composition
Lysis buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5 % NP40
RIPA buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.1 % SDS, 1 % Triton™ X-100, 1 % deoxycholate
Dilution/Wash buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA
2x SDS-sample buffer	120 mM Tris/Cl pH 6.8, 20 % glycerol, 4 % SDS, 0.04 % bromophenol blue, 10 % β -mercaptoethanol
Glycine-elution buffer	200 mM glycine pH 2.0
Neutralization buffer	1 M Tris pH 10.4