TurboGFP Nanoselector Agarose

Summary

Catalog No	014-101-002
Ligand	Anti-TurboGFP single domain antibody fragment (VHH, Nanobody)
Bead size	~ 40 μm
Reactivity	Recognizes TurboGFP selectively
Binding capacity	High binding capacity, 10 μL slurry bind about 20 μg of recombinant TurboGFP
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

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

Background

The dimeric green fluorescent protein TurboGFP is derived from the green fluorescent protein CopGFP of the copepod Pontellina plumata (Shagin et al., 2004). It possesses bright green fluorescence with excitation maximum at 482 nm and emission maximum at 502 nm. TurboGFP is a fast maturating protein: its fluorescent signal is visible earlier than other green fluorescent proteins. TurboGFP shares only about 20% sequence identity with jellyfish GFP variants. Therefore, most anti-GFP antibodies including the GFP-nanobody used in GFP Nanoselector Agarose do not bind to TurboGFP. TurboGFP is mainly intended for applications where fast appearance of bright fluorescence is crucial. It is specially recommended for cell and organelle labeling and tracking the promoter activity. Destabilized TurboGFP variant allows accurate analysis of rapid and/or transient events in gene regulation.

Application notes

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

Benefits

- Effective pulldown of TurboGFP-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 ~106- 107 cells.

1. Choice of lysis buffer:

* For cytoplasmic proteins, resuspend the cell pellet in 200 μL ice-cold Lysis buffer by pipetting up and down. Supplement Lysis buffer with protease inhibitor cocktail and 1 mM PMSF (not included).

* For nuclear/chromatin proteins, resuspend cell pellet in 200 μL 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 μL Dilution buffer supplemented with 1 mM PMSF and protease inhibitor cocktail (not included). If required, save 50 μL of diluted lysate for further analysis (input fraction).

Bead equilibration

1. Resuspend the beads by gently pipetting up and down or by inverting the tube. Do not vortex the beads!
2. Transfer 25 μL of bead slurry into a 1.5 mL reaction tube.
3. Add 500 μL ice-cold Dilution buffer.
4. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
5. Discard the supernatant.

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Protein binding

1. Transfer 25 μL of equilibrated beads to the tube of protein binding.
2. Rotate end-over-end for 1 hour at +4°C.

Washing

1. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
2. If required, save 50 μL of supernatant for further analysis(-flow-through/non-bound fraction).
3. Discard remaining supernatant.
4. Resuspend beads in 500 μL Wash buffer.
5. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. 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 μL 2x SDS-sample buffer.
3. Boil beads for 5 min at +95°C to dissociate immunocomplexes from beads.
4. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
5. Analyze the supernatant in SDS-PAGE.

Elution with Glycine-elution buffer

1.Remove the remaining supernatant.
2. Add 50–100 μL Glycine-elution buffer and constantly pipette up and down for 30 - 60 sec at +4°C.and down for 30 - 60 sec at +4°C.
3. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
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

Related products

Code Number	Product Description	Size	prices(¥)
019-101-002	GFP Nanoselector Agarose	0.25mL	1500
019-101-003	GFP Nanoselector Magnetic beads	0.25mL	1500
020-101-002	RFP Nanoselector Agarose	0.25mL	1500
020-101-003	RFP Nanoselector Magnetic beads	0.25mL	1500
013-101-002	mNeongreen Nanoselector Agarose	0.25mL	1500
014-101-002	TurboGFP Nanoselector Agarose	0.25mL	1500
015-101-002	MBP Nanoselector Agarose	0.25mL	1500
010-101-002	GST Nanoselector Agarose	0.25mL	1500
011-101-002	SNAP tag Nanoselector Agarose	0.25mL	1500
012-101-002	Halo Nanoselector Agarose	0.25mL	1500
003-101-002	HA tag Nanoselector Agarose	0.25mL	1500
004-101-002	c-His tag Nanoselector Agarose	0.25mL	1500
049-101-002	mWasabi Nanoselector Agarose	0.25mL	1500
017-101-002	TagFP Nanoselector Agarose	0.25mL	1500
025-101-002	Rabbit IgG Nanoselector Agarose	0.25mL	1500
001-101-002	Mouse IgG Nanoselector Agarose	0.25mL	1500
067-101-003	Streptavidin Magnetic beads	0.25mL	1500
100-100-100	Binding Control Nanoselector Agarose	1mL	800
100-100-200	Binding Control Magnetic beads	1mL	800

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