mScarlet Nanoselector Magnetic beads

Summary

Catalog No	028-101-003
Ligand	Anti-mScarlet single domain antibody fragment (VHH, Nanobody)
Bead size	~ 40 μm
Reactivity	mScarlet
Binding capacity	High binding capacity, 10 μL slurry bind about 20 μg of recombinant mScarlet.
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

mScarlet Nanoselector Magnetic beads have been specifically designed to bind mScarlet-fusion proteins. mScarlet Nanoselector Magnetic beads are based on small high-affinity recombinant single domain antibody covalently coupled to the surface of Magnetic beads. mScarlet Nanoselector Magnetic beads are ideal tools to isolate or purify mScarlet-fusion proteins fast and efficiently.

Background

The fluorescent protein mScarlet is a truly monomeric red fluorescent protein with record brightness, quantum yield (70%) and fluorescence lifetime (3.9 ns). mScarlet outperforms existing red fluorescent proteins as a fusion tag, and it is especially useful as a Förster resonance energy transfer (FRET) acceptor in ratiometric imaging.

For biochemical analysis including mass spectrometry and enzyme activity measurements these mScarlet-fusion proteins and their interacting factors need to be isolated fast and efficiently by immunoprecipitation using the mScarlet Nanoselector Magnetic beads. 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.

Application notes

Immunoprecipitation/ Co-IP
Mass spectrometry
On-bead enzyme assays
ChIP, RIP analysis

Benefits

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

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 ~10^6- 10^7 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

* 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).

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 μL of bead slurry into a 1.5 mL reaction tube.
3. Add 500 μL 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 μL of supernatant for further analysis(-flow-through/non-bound fraction).
3. Discard remaining supernatant.
4. Resuspend beads in 500 μL 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 μL 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 μL 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