MBP Nanoselector Magnetic beads

Summary

Catalog No	015-101-003
Ligand	Anti-MBP single domain antibody fragment (VHH, Nanobody)
Bead size	~ 2.8μm
Reactivity	Recognizes MBP fusion protein selectively
Binding capacity	High binding capacity, 10 μL slurry bind about 20 μg of recombinant MBP
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

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

Background

MBP is used to increase the solubility of recombinant proteins expressed in E. coli. In these systems, the protein of interest is often expressed as a MBP-fusion protein, preventing aggregation of the protein of interest. The mechanism by which MBP increases solubility is not well understood. In addition, MBP can itself be used as an affinity tag for purification of recombinant proteins. The fusion protein binds to amylose columns while all other proteins flow through.

Application notes

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

Benefits

- Effective pulldown of MBP-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 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).

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