Biotin Nanoselector Magnetic beads

Summary

Catalog No	078-101-003
Ligand	Anti-Biotin single domain antibody fragment (VHH, Nanobody)
Bead size	~ 2.8μm
Reactivity	Recognizes Biotin and biotinylated antibody, protein, peptides, oligonucleotides or solid matrices.
Binding capacity	High binding capacity, 10 μL slurry bind about 20 μg of biotinylated protein.
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

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

Background

Biotin is widely conjugated to proteins and antibodies for biochemical assays. Avidin (streptavidin)-biotin system is commonly used for many immunoassays such as ELISA, Flow Cytometry, Immunofluorescence, In Situ Hybridization, and Immunohistochemistry. Anti-Biotin antibody is a better alternative to avidins to minimize background and maximize signal intensity.

VHH are single-domain antibodies derived from the variable regions of heavy chain of Camelidae immunoglobulin. The size of VHH is extremely small(<15KDa) compared to other forms of antibody fragment, which significantly increase the permeability of VHH. Thus VHH is considered of great value for research, diagnostics and therapeutics.

Application notes

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

Benefits

- Effective pulldown of HA tag-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

Primary antibody binding(Optional steps)

1.take supernant of the cell lysis into another tube and then add primary antibody (2ug-4ug/sample).
2.rotate at +4°C overnight.

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