

# MBP Nanoselector Agarose

## Introduction

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. MBP Nanoselector Agarose is an affinity resin for immunoprecipitation of MBP fusion proteins. The MBP Nanoselector consists of an MBP Nanobody/ VHH coupled to agarose beads.

## Properties

<b>Ligand:</b>	Anti- single domain antibody (VHH, Nanobody)
<b>Affinity:</b>	KD=0.13 nM
<b>Reactivity:</b>	Recognizes -fusion proteins
<b>Binding capacity:</b>	2µg of recombinant per µL beads slurry
<b>Bead size:</b>	90 µm (cross-linked 4 % agarose beads)
<b>Formulation:</b>	50 % slurry in PBS containing 20 % Ethanol
<b>Storage conditions:</b>	Upon receipt store at +4°C. Do not freeze!
<b>Stability:</b>	Stable for 1 year upon receipt
<b>Buffer Compatibility:</b>	*Common buffer substances at pH 4 to 9 *2% Triton X-100, 2% NP-40, 0.1% SDS *3M NaCl, 1mM EDTA, 8 M urea, 3M Gua-Hcl

## 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<sup>6</sup> – 10<sup>7</sup> 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), MgCl<sub>2</sub> (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.

### Protein binding

1. Add diluted lysate to the equilibrated beads.

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  $\mu$ L Glycine-elution buffer and constantly pipette up 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 % $\beta$ -mercaptoethanol
Glycine-elution buffer	200 mM glycine pH 2.0
Neutralization buffer	1 M Tris pH 10.4

### Related products

Product name	Size	Cat#
GFP Nanoselector Agarose	0.5ml Resin 1ml Resin 5ml Resin	NBS01A-0.5ml NBS01A-1ml NBS01A-5ml
RFP Nanoselector Agarose	0.5ml Resin 1ml Resin 5ml Resin	NBS02A-0.5ml NBS02A-1ml NBS02A-5ml
turboGFP nanoselector Agarose	0.5ml Resin 1ml Resin 5ml Resin	NBS04A-0.5ml NBS04A-1ml NBS04A-5ml
mNeonGreen Nanoselector Agarose	0.5ml Resin 1ml Resin 5ml Resin	NBS03A-0.5ml NBS03A-1ml NBS03A-5ml
HA tag Nanoselector Agarose	0.5ml Resin 1ml Resin 5ml Resin	NBS09A-0.5ml NBS09A-1ml NBS09A-5ml
MYC tag Nanoselector Agarose	0.5ml Resin 1ml Resin 5ml Resin	NBS12A-0.5ml NBS12A-1ml NBS12A-5ml
GST Nanoselector Agarose	0.5ml Resin 1ml Resin 5ml Resin	NBS06A-0.5ml NBS06A-1ml NBS06A-5ml
MBP Nanoselector Agarose	0.5ml Resin 1ml Resin 5ml Resin	NBS05A-0.5ml NBS05A-1ml NBS05A-5ml
Halo nanoselector Agarose	0.5ml Resin 1ml Resin 5ml Resin	NBS08A-0.5ml NBS08A-1ml NBS08A-5ml
SNAP/CLIP nanoselector Agarose	0.5ml Resin 1ml Resin 5ml Resin	NBS07A-0.5ml NBS07A-1ml NBS07A-5ml