Amylose Magnetic Beads

1-800-632-7799
info@neb.com
www.neb.com

E8035S

25 mg (2.5 ml) **Lot: 0081604** **Exp: 4/19**

10 mg/ml **Store at 4°C (Do not freeze)**

Description: An affinity matrix for the small-scale isolation and purification of MBP-fusion proteins. Amylose is covalently coupled to a paramagnetic particle through a linkage that is stable and leak resistant over a wide pH range. This permits the isolation of MBP-fusion proteins from cell culture supernatants. Immobilized fusion proteins can be used in subsequent experiments to capture target proteins from crude cell lysates that may interact with the immobilized fusion protein.

Supplied as a 10 mg/ml suspension in water containing 20% ethanol.

Support Matrix: Amylose Magnetic Beads are 10 µm superparamagnetic microparticles.

Binding Capacity: 1 mg of Amylose Magnetic Beads will bind 5–10 µg of MBP-fusion protein.

Protocol:

MBP Column Binding Buffer:
200 mM NaCl
20 mM Tris-HCl
1 mM EDTA
1 mM DTT
(pH 7.4 @ 25°C)

Isolation of MBP-fusion protein using Amylose Magnetic Beads:

The following protocol is for the isolation of MBP-fusion protein from 200–500 µl cell culture supernatant.

1. Vortex and thoroughly suspend magnetic beads.
2. Aliquot 100 µl of bead suspension to a sterile microcentrifuge tube.
3. Add 500 µl of MBP column buffer and vortex to suspend. Apply magnet for 30 seconds, to pull beads to the side of the tube and decant supernatant. Repeat wash.
4. Add 200–500 µl of cell culture supernatant to the beads.
5. Mix thoroughly and incubate at 4°C with agitation for 1 hour.
6. Apply magnet and decant supernatant.
7. Wash beads three times as in step 3 above.

At this point the purified MBP-fusion can be eluted from the beads or used directly for capture of target proteins.

MBP-Fusion Elution:

1. Add 50 µl of MBP column buffer containing 10 mM maltose (elution buffer) to the bead pellet, vortex and incubate for 10 minutes at 4°C with agitation.
2. Apply magnet and pipet eluted MBP-fusion protein into a clean microcentrifuge tube.
3. Add an additional 50 µl of elution buffer to the beads and repeat elution step. Pool elution supernatants.

Note: Efficiency of elution can be checked by eluting any protein that remains bound to the Amylose Magnetic Beads with 50 µl of SDS-PAGE gel loading buffer and running 15 µl on denaturing protein gel.

NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

CERTIFICATE OF ANALYSIS

Amylose Magnetic Beads

1-800-632-7799
info@neb.com
www.neb.com

E8035S

25 mg (2.5 ml) Lot: 0081604 Exp: 4/19

10 mg/ml Store at 4°C (Do not freeze)

Description: An affinity matrix for the small-scale isolation and purification of MBP-fusion proteins. Amylose is covalently coupled to a paramagnetic particle through a linkage that is stable and leak resistant over a wide pH range. This permits the isolation of MBP-fusion proteins from cell culture supernatants. Immobilized fusion proteins can be used in subsequent experiments to capture target proteins from crude cell lysates that may interact with the immobilized fusion protein.

Supplied as a 10 mg/ml suspension in water containing 20% ethanol.

Support Matrix: Amylose Magnetic Beads are 10 µm superparamagnetic microparticles.

Binding Capacity: 1 mg of Amylose Magnetic Beads will bind 5–10 µg of MBP-fusion protein.

Protocol:

MBP Column Binding Buffer:
200 mM NaCl
20 mM Tris-HCl
1 mM EDTA
1 mM DTT
(pH 7.4 @ 25°C)

Isolation of MBP-fusion protein using Amylose Magnetic Beads:

The following protocol is for the isolation of MBP-fusion protein from 200–500 µl cell culture supernatant.

1. Vortex and thoroughly suspend magnetic beads.
2. Aliquot 100 µl of bead suspension to a sterile microcentrifuge tube.
3. Add 500 µl of MBP column buffer and vortex to suspend. Apply magnet for 30 seconds, to pull beads to the side of the tube and decant supernatant. Repeat wash.
4. Add 200–500 µl of cell culture supernatant to the beads.
5. Mix thoroughly and incubate at 4°C with agitation for 1 hour.
6. Apply magnet and decant supernatant.
7. Wash beads three times as in step 3 above.

At this point the purified MBP-fusion can be eluted from the beads or used directly for capture of target proteins.

MBP-Fusion Elution:

1. Add 50 µl of MBP column buffer containing 10 mM maltose (elution buffer) to the bead pellet, vortex and incubate for 10 minutes at 4°C with agitation.
2. Apply magnet and pipet eluted MBP-fusion protein into a clean microcentrifuge tube.
3. Add an additional 50 µl of elution buffer to the beads and repeat elution step. Pool elution supernatants.

Note: Efficiency of elution can be checked by eluting any protein that remains bound to the Amylose Magnetic Beads with 50 µl of SDS-PAGE gel loading buffer and running 15 µl on denaturing protein gel.

NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

CERTIFICATE OF ANALYSIS