Chitin Magnetic Beads

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

E8036S 00817062006 1

E8036S

5 ml **Lot: 0081706**

Store at 4°C (Do not freeze) **Exp: 6/20**

Description: An affinity matrix for the small-scale isolation of target proteins fused to a chitin binding domain (CBD) (1). Chitin beads have been prepared having a magnetite core. This permits the magnetic isolation of CBD-fusion proteins from cell culture supernatants; after which, the matrix can be regenerated without loss of binding capacity. Immobilized fusion proteins can be used in subsequent experiments to capture target proteins from crude cell lysates that interact with the immobilized fusion protein.

LABORATORY REAGENT – FOR RESEARCH USE ONLY

Supplied as a 50:50 (v:v) suspension in water containing 20% ethanol.

Support Matrix: Chitin Magnetic Beads are approximately 50–70 µm paramagnetic microparticles.

Binding Capacity: 100 µl of Chitin Magnetic Beads will bind 30–50 µg of CBD fusion protein.

Protocol:

CBD Column Binding Buffer:
500 mM NaCl
20 mM Tris-HCl
1 mM EDTA
0.05% Triton X-100
(pH 8.0 @ 25°C)

Isolation of CBD-fusion protein using Chitin Magnetic Beads:

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

1. Vortex and thoroughly suspend magnetic beads.
2. Aliquot 50 µl of bead suspension to a sterile microcentrifuge tube.
3. Add 500 µl CBD 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 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 CBD-fusion can be eluted from the beads or used directly for capture of target proteins.

CBD-Fusion Cleavage: To determine the required method of intein-fusion cleavage please refer to applicable NEB IMPACT Manual.

Notes: Efficiency of elution can be checked by eluting any protein that remains bound to the chitin magnetic beads with 50 µl of SDS-PAGE gel loading buffer and running 15 µl on a denaturing protein gel.

Reference:

1. Chong, S. et al. *Gene* 192, 271–281.

CERTIFICATE OF ANALYSIS

Chitin Magnetic Beads

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

E8036S 00817062006 1

E8036S

5 ml Lot: 0081706

Store at 4°C (Do not freeze) Exp: 6/20

Description: An affinity matrix for the small-scale isolation of target proteins fused to a chitin binding domain (CBD) (1). Chitin beads have been prepared having a magnetite core. This permits the magnetic isolation of CBD-fusion proteins from cell culture supernatants; after which, the matrix can be regenerated without loss of binding capacity. Immobilized fusion proteins can be used in subsequent experiments to capture target proteins from crude cell lysates that interact with the immobilized fusion protein.

LABORATORY REAGENT – FOR RESEARCH USE ONLY

Supplied as a 50:50 (v:v) suspension in water containing 20% ethanol.

Support Matrix: Chitin Magnetic Beads are approximately 50–70 µm paramagnetic microparticles.

Binding Capacity: 100 µl of Chitin Magnetic Beads will bind 30–50 µg of CBD fusion protein.

Protocol:

CBD Column Binding Buffer:
500 mM NaCl
20 mM Tris-HCl
1 mM EDTA
0.05% Triton X-100
(pH 8.0 @ 25°C)

Isolation of CBD-fusion protein using Chitin Magnetic Beads:

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

1. Vortex and thoroughly suspend magnetic beads.
2. Aliquot 50 µl of bead suspension to a sterile microcentrifuge tube.
3. Add 500 µl CBD 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 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 CBD-fusion can be eluted from the beads or used directly for capture of target proteins.

CBD-Fusion Cleavage: To determine the required method of intein-fusion cleavage please refer to applicable NEB IMPACT Manual.

Notes: Efficiency of elution can be checked by eluting any protein that remains bound to the chitin magnetic beads with 50 µl of SDS-PAGE gel loading buffer and running 15 µl on a denaturing protein gel.

Reference:

1. Chong, S. et al. *Gene* 192, 271–281.

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