

Isolation of CBD-fusion protein using Chitin Magnetic Beads

Overview

Introduction

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

Buffers

CBD Column Binding Buffer

Protocol

1. Vortex and thoroughly suspend magnetic beads.
2. Aliquot 50 μ l of bead suspension to a sterile microcentrifuge tube.
3. Add 500 μ l 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 μ l 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 μ l of SDS-PAGE gel loading buffer and running 15 μ l on a denaturing protein gel.

*** 1X CBD Column Binding Buffer:**

NaCl 500 mM, Tris-HCl 20 mM, EDTA 1 mM, Tween-20 0.1%, pH 8 @25°C.