



Chitin binding assay using chitin magnetic beads (NEB)

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MATERIALS

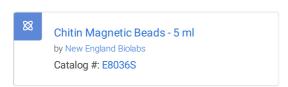
NAME Y	CATALOG #	VENDOR V
Chitin Magnetic Beads - 5 ml	E8036S	New England Biolabs
STEPS MATERIALS		
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1 Make the following buffer:

1 X CBD Column Binding Buffer

Tris-HCl	20 mM Adjust pH to 8 using HCl	(2,42 g/L)
NaCl	500 mM	(29,22 g/L)
EDTA	1 mM	(0,292 g/L)
Tween-20	0.05% v/v	500 ul/ 1 L

Gently mix the beads solution and resuspend thoroughly.



- 2 Aliquot 50 ul of beads solution into a 2 ml microfuge tube
- 3 Apply a magnet for 30 seconds to pull the beads to one side. Remove the excess liquid

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Wash the beads 3 times with 500 ul of 1x CBD Column Binding Buffer. Apply magnet and remove the liquid.

Load 200-500 ul of cell supernatant with the beads.
Note: when making dilutions of the supernatant, dilute in CBD Column Binding Buffer.

Mix thoroughly and incubate for 1 hour at 4°C at constant agitation.

1h

Apply magnet for 30 seconds and remove supernatant with a pipette. Save the supernatant.

Wash beads 3 times with 500 ul of 1x CBD Column Binding Buffer. Apply magnet for 30 seconds and pipette off liquid. Save the wash fractions.

To remove proteins from the beads, boil beads for 5 minutes in 50 ul SDS-Page sample buffer at 95 °C

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Run fractions on a protein gel

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