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Chitin binding assay using chitin magnetic beads (NEB)

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Protocol status: In development
We are still developing and optimizing this protocol

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Materials

MATERIALS

Chitin Magnetic Beads - 5 ml **New England Biolabs Catalog #**E8036S

STEP MATERIALS

Chitin Magnetic Beads - 5 ml **New England Biolabs Catalog #**E8036S

Protocol materials

Chitin Magnetic Beads - 5 ml **New England Biolabs Catalog #**E8036S

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.

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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 3s

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

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

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

7 Apply magnet for 30 seconds and remove supernatant with a pipette. Save the supernatant. 3s

8 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. 3s

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

10 Run fractions on a protein gel