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GFP pull down assay

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes GFP pull down assay.

Attachments

PDF

754-1922.pdf

43KB

Materials

Materials

- GFP-Trap agarose beads (Chromotek)
- dH_2O
- Protein Loading dye

Bead assay buffer

	A	B
	Tris-HCl pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM

GFP pull down assay

2h 5m

1 Mix GFP-tagged TBK1 with 20 µL of equilibrated GFP-Trap agarose beads (Chromotek) at a final concentration of [M] 1 micromolar (µM). Make sure to wash beads 2x in dH2O before washing with bead assay buffer to equilibrate the beads adding the protein to the beads.

2 To this end, wash 20 µL of beads twice with dH_2O and equilibrate with bead assay buffer.

3 Resuspend the beads in 40 µL bead assay buffer, to this add GFP-TBK1 at a final concentration of [M] 5 micromolar (µM).

4 Incubate the beads with GFP-TBK1 for 01:00:00 at 4 °C at a horizontal tube roller.

1h

5 Wash the beads three times to remove unbound GFP-tagged bait protein.

6 Prepare protein master mixes with prey protein in bead assay buffer at the following concentrations:

- mCherry-OPTN (1 µM),
- mCherry-NDP52 (1 µM),
- GST-NAP1 (1-10 µM).

7 Add the protein master mixes to the beads and incubate for 01:00:00 at 4 °C at a horizontal tube roller.

1h

8 Wash the beads three times to remove unbound proteins, remove any supernatant from the beads and resuspend the beads in 60 µL of 1x Protein Loading dye, and heat-inactivate at 95 °C for 00:05:00.

5m

9 Analyze the samples by SDS-PAGE and Coomassie staining as described above.