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GFP pull down assay

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ABSTRACT

This protocol describes GFP pull down assay.

ATTACHMENTS

754-1922.pdf

MATERIALS

Materials

- GFP-Trap agarose beads (Chromotek)
- dH₂O
- Protein Loading dye

Bead assay buffer

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

OPEN ACCESS



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

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2h 5m

the protein to the beads.

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

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2 To this end, wash \blacksquare 20 μ L of beads twice with dH₂O 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)



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



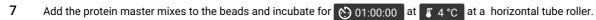


5 Wash the beads three times to remove unbound GFP-tagged bait protein.



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

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







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



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

