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# GST Bead pulldown Assay

Forked from WIPI2d Coprecipitation Assay

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ABSTRACT

GST Pulldown Assay for recruitment of bait proteins to GST labeled prey proteins. Prey proteins can be purified or from lysate.

PROTOCOL CITATION

Adam Yokom 2022. GST Bead pulldown Assay. **protocols.io** https://protocols.io/view/gst-bead-pulldown-assay-ccwksxcw

FORK NOTE

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Forked from WIPI2d Coprecipitation Assay, lmstrong

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1 Homogenize cell pellet with GST tagged protein in 0.5 ml of lysis buffer/protease inhibitors/1% TritonX-100. Clarify lysate by centrifugation at 40,000g for 15 min

2 Equilibrate 30uL of Glutathione Sepharose beads (GE Healthcare) into pulldown buffer. To do this, pipette 60uL of 50% slurry into a 1.7uL eppy. Add >500mL of wash buffer. Slow spin to pellet resin. ~1000rpm for 1 minute should be good. Repeat X3

3 Mix clarified lysate and washed GST resin together.

4 Add 1-10 µM purified protein (add buffer of 25 mM HEPES pH 7.5, 150mM NaCl, 1mM MgCl2, 1mM TCEP to final volume of 200 uL). Alternatively, add 10mL of HEK293GnTi lysate.

5 Let rock at 4C overnight. Wash x3 with buffer: 25 mM HEPES pH 7.5, 150mM NaCl, 1mM MgCl2, 1mM TCEP

6 Elute washed resin with 50uL buffer: 25 mM HEPES pH 7.5, 150mM NaCl, 1mM MgCl2, 1mM TCEP + 25 mM glutathione

7 Mix 17 uL eluent with 3 uL SDS-Loading dye. Heat samples for 5min @ 60C.

8 Run beads on SDS-PAGE gel and stain with Coomassie.