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GST Bead pulldown Assay

Forked from WIPI2d Coprecipitation Assay

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We use this protocol and it's working

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Disclaimer

Abstract

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

Troubleshooting

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.