GST pulldown assay

Protein-protein interaction, cultured cells

Ingredients

1) Lysis buffer:
25mM HEPES (or Tris-HCl) pH 7.5, 150mM NaCl, 10mM $MgCl_2$, 10% glycerol, 1% NP40
Notes – protease/phosphatase inhibitors added, salt concentration can be increased up to 0.5M to reduce non-specific binding, detergent can be adjusted (for example, can substitute a 1% Triton X-100, 0.1% SDS, 0.1% deoxycholate detergent formulation)
2) Glutathione Sepharose beads (GE Healthcare) – washed in 1xPBS, 50% 1xPBS slurry
3) 1x PBS (sterile)
4) SDS-PAGE loading buffer (Laemmli protein sample buffer) - 0.2M Tris-HCl pH 6.8, 40% Glycerol, 4% SDS, 0.2% Bromophenol Blue (BPB)(0.032%); add β-Mercaptoethanol to 5% (fresh – will be okay for ~1 month)

Steps

1) Transfect cells with plasmids (typically 0.5 to 1ug/construct per well, 6-well plate at ~70% confluency); GST proteins are expressed using the pRK5m, or pCDNA3 mammalian expression plasmids.
Note: If using HEK cells, seed onto poly-D lysine (or other adhesion substrate) plates
2) Next day, wash cells once with 1xPBS (1ml) and scrape cells into 150uls lysis buffer
3) Transfer to 1.5ml Eppendorf tube, pipette up and down 10x to lyse the cells
4) Spin down cell debris, max speed at 4°C in a microcentrifuge.
5) Discard pellet, and transfer supernatant to a fresh tube
6) Save a portion of the lysate (input sample), measure the protein concentration
7) Pulldown – add ~25uls 50% glutathione (GSH) bead slurry, mix and rock overnight at 4°C (or minimum 3h at 4°C)
8) Spin down the beads, take off the supernatant, wash with 0.5 to 1 ml lysis buffer; repeat 3x
9) Resuspend the beads in SDS-PAGE loading buffer with DTT or 5% β-Mercaptoethanol (must be relatively fresh!), boil 5 mins
10) Run gels on an SDS-PAGE gel, and immunoblot