GST-PULL DOWN FOR PROTEIN INTERACTION STUDIES

1. GST-protein fusions are immobilized on glutathione-sepharose beads by incubating the purified GST-protein fusions with glutathione-sepharose beads (Pharmacia) (pre-washed with TEN100 [20 mM Tris, pH 7.4, 0.1 mM EDTA and 100 mM NaCl] 4 times and equilibrated in TEN100) at 4°C nutating for 1 hour. *In a similar fashion, bind purified GST to beads for use as a negative control.*

2. Wash the beads four times with 100 volumes of TEN100 to remove unbound material, resuspend in an equal volume of TEN100, and store at 4°C.

3. Incubate whole cell extract of choice or purified protein(s) with the immobilized GST or GST-protein fusion at 4°C nutating for 1 hr.

4. Wash the beads four times with 100 bed volumes of NETN buffer (0.5% NP40, 0.1 mM EDTA, 20 mM Tris, pH 7.4, 300 mM NaCl) for whole cell extract, or TEN buffer (20 mM Tris, pH 7.4, 0.1 mM EDTA and 100 mM NaCl) for purified protein(s).

   The washing conditions are dependent on the protein which is analyzed. Sometimes buffer containing high salt is necessary to attain low background. A good first choice is NETN300, which contains 300 mM NaCl.

5. The bound proteins are eluted by boiling in sample buffer and visualized using Coomassie blue staining or Western blot analysis.

Reference:

Frangioni, J. V., & Neel, B. G. (1993). *Analytical Biochemistry* **210**, 179-187.