GST Pulldown – for 6 well plate

Transfect cells with transfection reagent of choice.

1. Wash cells with 500 μl 1x PBS
2. Lyse cells by adding 350 μl IP/Lysis Buffer (with 0.1% NP-40 and add PIC fresh)
3. Incubate on ice for 30 min., vortexing every 10 min.
4. Centrifuge 14,000 rpm, 4ºC, 15 min.
5. Save 80 μl lysate for total lysate and add to 80 μl 2x Tris/tricine sample buffer
6. Prepare Glutathione Sepharose beads (20 μl beads/ sample) by washing total amount of beads 2x with 500 μl 1x PBS
    a. Centrifuge 500 x g for 5 min. to pellet beads
    b. Pipet off buffer, then add 1x PBS and proceed with washes
    c. Centrifuge in between washes 500 x g for 5 min.
    d. Pipet off supernatant
    e. After final wash, resuspend beads in 1x PBS (20 μl 1x PBS/ number of samples)
    f. Aliquot 20 μl washed beads into 1.5 ml microcentrifuge tubes
7. Add remaining lysate to 1.5 ml microcentrifuge tube containing 20 μl Glutathione Sepharose beads
8. Rotate in cold room for 2 hours
9. Centrifuge 500 x g for 5 min., 4ºC
10. Wash 4x with 250 μl IP/Lysis buffer (with 0.1% NP-40 and PIC fresh)
    a. Centrifuge in between washes 500 x g, 4ºC for 5 min.
    b. Pipet off supernatant
11. After final wash, pipet off most of the supernatant and add 20 μl 2x Tris/tricine sample buffer to a total of approximately 40 μl.

Lysis Buffer:
137 mM NaCl
10 mM Tris, pH 7.4
1% NP-40

2x Tricine Sample Buffer (for 50 ml):
5 ml 1M Tris, pH 6.8
12 ml Glycerol
4 g SDS
1.55 g DTT
10 mg Coomassie Blue R250
to 50 ml with dH_2O