Pulldown of GST fusion mixed with extract of BL21(DE3)/codon+ expressing protein of interest

The following protocol is for mixing a bacterial lysate with up to 4 GST proteins; scale accordingly

Day 1:
1. Start 1.5 mL culture of BL21 strain in LB+amp+chloramphenicol; shake at 37˚C overnight

Day 2:
2. Dilute BL21 culture 1:100 in 50 mL LB+amp+chloramphenicol
3. Shake culture at 37˚C until OD600 = 0.5, then place culture flask in ice-water bath for 5 min
4. Transfer culture flask to 20˚C shaker and add IPTG @ final of 0.5 mM; shake overnight

Day 3:
5. Spin out cells at 5,000 rpm for 10 min; decant and aspirate supernatant
6. Resuspend pellet in 2 mL ice-cold lysis buffer
7. Store on ice 30 min
8. During incubation in step 7, prepare GST-sepharose columns:
   a. Equilibrate glutathione-sepharose beads:
      • need 20 μL slurry for each pulldown; prepare 2 extra aliquots
      • combine total slurry in 1 eppi tube; add ice-cold H2O
      • spin 2 min at 10,000 rpm at 4˚C; aspirate without getting close to beads
      • add 1 mL ice-cold PBS, spin, and aspirate; repeat once more
      • resuspend beads in ice-cold PBS (1 aliquot/100 μL)
   b. Bind GST proteins to beads:
      • aliquot equilibrated beads to siliconized eppi tubes on ice
      • add 500 μL ice-cold PBS to each aliquot
      • add specific GST protein (usually 5 μg) to each aliquot
      • rotate at 4˚C for 40 min
   c. Wash GST columns:
      • spin 2 min at 10,000 rpm at 4˚C; aspirate without getting close to beads
      • add 1 mL ice-cold PBS, spin, and aspirate; repeat twice more
      • resuspend beads in ice-cold PBS (1 aliquot/100 μL)
      • maintain GST columns on ice until step 14
9. Sonicate lysate for 20 seconds at 15W; return to ice
10. Transfer lysate to 2 eppi tubes and spin 5 min at full speed at 4˚C
11. Repeat step 9
12. Transfer supernatant to Beckman-eppi tubes and spin 10 min at 55,000 rpm (100,000 x g) at 4˚C
13. Mix 20 μL of supernatant (1% of total lysate) with 20 μL 2X sample buffer; boil 5 min; load 10 μL (0.25% of total lysate)
14. Divide remaining supernatant into 4 equal aliquots; add each aliquot to specific GST column prepared in step 8
15. Rotate at 4˚C for 40 min
16. Spin 2 min at 10,000 rpm at 4˚C; aspirate without getting close to beads
17. Add 1 mL ice-cold PBS + 0.1% TX-100, spin, and aspirate; repeat twice more
18. Add 1 mL ice-cold PBS (without TX-100), spin, and aspirate; repeat once more
19. Dry beads in speed-vac
20. Add 50 μL sample buffer; boil; load 10 μL (5% of total lysate)

Lysis buffer