SF9 Protein Purification

1. Prepare Fresh Buffers

Lysis Buffer: 50 mM Tris (pH 7.7)
150 mM KCl
0.1% Triton-X 100
1X Protease Inhibitors (1000x= 10mg/ml leupeptin, pepstatin A, chymostatin) , O.A. (1000x = 1mM)

Wash Buffer: 50 mM Tris (pH 7.7)
300 mM KCl

Elution Buffer: 50mM Tris (pH 7.7)
300 mM KCl
+ 250 mM Imidazole for His Tag Fusions
OR + 10 mM Glutathione for GST Fusions

pH Elution Buffer back to 7.7 after addition of
****imidazole or glutathione****

Protein Storage Buffer:
50 mM Tris (pH7.7)
100 mM KCl
1 mM DTT
10% Glycerol

2. Lyse Cells

Add ~20 ml lysis buffer/liter of SF9 cells harvested, sit on ice for 20 minutes. Dounce cells 30 times, or sonicate for 2 minutes.

*Take sample of lysate for SDS PAGE gels

3. Prepare Supernatant

Spin lysate at top speed (~18,000 rpm) for 1 hour

*Take sample of supernatant for SDS PAGE gels

4. Equilibrate 2 ml solid beads/liter of SF9 cells by washing with lysis buffer.

5. Add beads to lysate, incubate in cold room, rotating, for 2 hours

6. Start equilibrating QuickChange PD10 Columns with protein storage buffer (need 5X flow through!!!!)

7. Wash beads 5X with 25 ml Wash Buffer/Wash on column

   *Take sample of first Wash for SDS PAGE gels

8. Collect 2 elutions with 2.5 ml elution buffer each, Let each elution incubate with beads for 15 minutes before collecting elution.

   *Take sample of elutions for SDS PAGE gels

9. Immediately use QuickChange column to change buffer on elutions to protein storage buffer

   *Take samples of PD10 Quickchange column elutions

10. Run SDS PAGE gel on fractions to determine purification quality and quantity (LYSATE, SUPERNATANT, WASH, ELUTIONS, PD10 Elutions)