How to Determine Whether Your Protein is Soluble or Insoluble

Since disulfide bridges do not form in the cytoplasm of prokaryotic cells, many proteins are not able to fold properly and may form insoluble aggregates called "inclusion bodies".

The easiest way to determine if your protein is forming inclusion bodies is to lyse the cells; spin out the insoluble fraction then run both the supernatant (soluble) and the pellet (insoluble) fraction on SDS-PAGE.

Glass Bead MethodP

RE-TREATMENT OF GLASS BEADS

1. Add 1mL of concentrated HCl to 500mg beads in a microfuge tube.
2. After 5 min. wash 5 times with dd H2O (1mL each).
3. Then wash glass beads twice with 1 mL of TDEP buffer.
4. Remove as much liquid as possible, then add TDEP buffer to 1 mL total volume.

NOTE: Add: 0.1mm glass beads. Important that ratio of glass beads to cells is 1mg beads/mg cells or more.

GLASS BEAD METHOD FOR ISOLATING TOTAL BACTERIA PROTEINS

1. Weigh an empty tube and add 1.5ml exponential culture (ie: O.D. 600 ~0.6).
2. Pellet cells and wash with TDEP buffer. *
3. Weigh the washed cells and resuspend in 200(L of ice-cold TDEP buffer.
4. Add the cells to 1 mg glass beads per mg of cells.
5. Vortex for 30 sec. with 30 sec. incubations on ice between vortexing , for a total of 4 times.
6. Remove the cell debris and DNA by centrifugation for 10 min. in microfuge.
7. Transfer the supernatants to fresh tubes and store at -20(C.

* TDEP Buffer

- 10mM Tris. Cl, pH 7.4
- 0.5mM DTT
- 1.0mM EDTA
- 35(g/ml PMSF (0.2mM)

Freeze/Thaw Method (native conditons)

1. Spin down 1.5mL of induced cells (O.D. 0.6-3)

2. Resuspend cells in 40mM phosphate, 500mM NaCl pH 8 buffer.
3. Add lysozyme to 100ug/mL and incubate for 20 min at 30 degree C.
4. Flash freeze the crude lysate in liquid nitrogen, then thaw at 37 degree C.
5. Repeat step 4 two more times--this should lyse the cells.
6. Treat the lysate with DNase/RNase at 5ug.mL final for 15 min at 30 degree C.
7. Spin down insoluble debris at 3000Xg for 15 min.
8. Remove supernatant to fresh tube--soluble proteins will be in this fraction--run 10uL on SDS-PAGE.
9. Resuspend pellet in 150uL of 2% SDS to solubilize. Run 10uL of this on SDS-PAGE--insoluble proteins will be in this fraction.