Inclusion body preparation

Purification of inclusion bodies from *E.coli*. For protein production in *E.coli*, see a separate protocol. For a good review on refolding proteins from inclusion bodies see Rudolph and Lilie (1996).

1. The cells should be well suspended in appropriate buffer. To facilitate lysis and inclusion body purification, add 0.5–1.0 % Triton X-100. Some EDTA and DTT, up to 50 mM, should be used in all subsequent steps to keep disulfides reduced, this is important for all proteins. It might even be a good idea to degas all the buffers beforehand.

2. If the cells have been frozen, thaw them thoroughly before lysis.

3. The cells can be lysed with either French press or sonication. With French press the cells should be passed at least twice through to ensure complete shearing of the genomic DNA. Sonication conditions should be optimised depending on the sonicator, tip, amount of cells in per volume of lysis buffer etc. As a starting point use four 20 second cycles with 30 second cooling in between for a 25 ml sample. Be careful to break all the cells or your inclusion body preparation will be heavily contaminated with other proteins. Keep a small sample of the total lysate for SDS-PAGE.

4. Spin the sample ca. 20 min 15 000 rpm. Take a small sample of the supernatant for SDS-PAGE and discard the rest .

5. Resuspend the inclusion body pellet in a small volume of buffer containing 1-2 % Triton X-100. This should solubilise membranes and membrane proteins. The better you resuspend the pellet, the better the result will be in the end. A short sonication (3 x 10 seconds) is very helpful during each wash step. Not only will it help to resuspend all the inclusion bodies, but it will also break unbroken cells and shear DNA. Fill the centrifuge tube with the same buffer (incl. Triton X-100) and vortex well.

6. Centrifugation etc. as in 4.

7. Resuspend the pellet as in 5., but now to a buffer containing 1 M NaCl. This will remove lot of the DNA and RNA from the pellet. If your protein is very positively charged (eg. DNA or heparin binding), you might find it useful to use even higher concentration of salt in the wash.

8. Centrifugation etc. as in 4.

9. Last wash of the pellet will be with the buffer alone. Take a sample of the resuspended pellet for SDS-PAGE (You won't be able to run the solubilised sample from the next step on the gel since SDS aggregates in high GndHCl concentrations).

10. Centrifugation etc. in 4. Discard supernatant and keep the pellet.

11. Solubilise the purified inclusion bodies into 6 M guanidine HCl (GndHCl) or 8 M urea with appropriate buffer and 5-100 mM DTT.[1] Again, resuspending and homogenising the pellet in a small volume first will help the solubilisation. Some inclusion bodies are very difficult to solubilise and you might want to leave them to dissolve over-night. If the solution becomes very viscous after adding the denaturant, you have probably failed to lyse all the cells and they are now being broken and have released their genomic DNA (and all the proteins). Brown colour can be an indication that you have not kept the inclusion bodies with cysteine rich proteins in reducing enough conditions. The solubilised inclusion bodies should give complete colourless solution.

12. Centrifuge as before, but keep the supernatant this time.

(Optional) Especially with disulfide rich proteins it is necessary to control also the redox conditions, and therefore it is advisable to remove DTT from the sample before refolding. An easy and quick way to do this is to use Pharmacia HiPrep 26/10 Desalting column. For this, prepare fresh [2] 500 ml of 6M urea (or GndHCl if you prefer), 20 mM Tris pH 8.0, 0.5 mM EDTA and equilibrate the column with this. Load 10 ml of the sample to the column at a time and collect the protein fraction which is now in the urea buffer. The sample will be diluted by approximately 1.4-fold. Wash the column sufficiently between loadings to remove all the GndHCl, DTT etc. from the previous run.

13. Run a UV spectrum of the sample. You should get nice protein spectrum with a maximum at 280 nm and minimum at ca. 250 nm—if not, your sample is most probably contaminated with DNA/RNA.

14. Run a gel to check for the success of the inclusion body preparation. The purer the final sample is, the better you have done.

15. Proceed to refolding.

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References

Rudolph, R. and Lilie, H. (1996) In vitro refolding of inclusion body proteins. *Faseb J*, **10**:49–56.

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[1] For cysteine rich proteins you might need to titrate the minimum DTT concentration required for full reduction. And even more importantly to avoid oxidation of cysteines to form sulfenic and sulfonic acids.

[2] Urea breaks to isocyanate which can modify lysine residue. The process is fastest at high pH, and virtually non-existent below pH 4, so you might wish to make the urea solution for example in 10mM HCl. In any case, use only freshly prepared urea