



## INSTRUCTIONS

# Protein inclusion body preparation and solubilization

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The following protocols of protein inclusion body preparation and solubilization are recommended.

### **Inclusion body isolation:**

1. Resuspend the cell pellet in 20 ml of cell lysis buffer (20mM Tris-HCl, pH 8, 100 mM NaCl, 2 mM DTT, 2 mM EDTA) for each liter of culture. Increase the volume proportionally for cell pellets from more than 1 L of culture.
2. Break the cells by passing the cell suspension French Press twice.
3. Centrifuge the broken cell suspension at 20,000 rpm for 20 min.
4. The crude inclusion bodies are in the pellet.

### **Inclusion body purification:**

1. Resuspend the crude inclusion bodies in the cell lysis buffer plus 1 % Triton-100 by stirring at 4C for 1 to 2 hours.
2. Centrifuge the suspension at 20,000 rpm for 20 min. Discard the supernatant.
3. Resuspend again the pellet in the cell lysis buffer without Triton.
4. Centrifuge the suspension at 20,000 rpm for 20 min. Discard the supernatant. The pellet is the purified inclusion bodies.

### **Inclusion body solubilization:**

If it is a membrane protein, use urea to solubilize the protein. If it not a membrane protein, use 8 M urea or 6 M guanidine hydrochloride to solubilize the protein.

1. Estimate the amount of protein in the purified inclusion bodies. Add the volume of the solubilization buffer (20 mM TrisHCl, pH 8.0, 6 M guanidine hydrochloride (or 8 M urea), 10 mM DTT) to make about 10 mg/ml protein concentration. The solubilization is performed by stirring the inclusion bodies with the solubilization buffer at room temperature for 2 to 4 hours. Most of the pellet should be solubilized.
2. Centrifuge the solubilization material at 30,000 rpm for 45 min. Save the supernatant as the solubilized inclusion bodies.

### **Protein folding kits using solubilized inclusion bodies:**

#### **Soluble proteins:**

Spin-column protein folding screen kit  
96-well protein folding plate

Catalog number: SFC01-10  
Catalog number: PFS096

#### **Membrane proteins:**

Dilution Membrane Protein Folding Screen Kit  
Spin-column Membrane Protein Folding Screen Kit

Catalog number: MPS10-20  
Catalog number: MFC01-20