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Lysis of Mammalian and Sf9 Cells

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Abstract

Use a French press to disrupt mammalian or Sf9 cells and generate a clarified lysate for subsequent use in protein purification.

1. THEORY

There are numerous methods to lyse cells including osmotic shock, freeze-thawing, and manual disruption such as Dounce homogenization. Lysis with a French Pressure Cell offers many advantages over these other techniques including speed, efficiency of cell disruption, and compatibility with numerous buffer conditions. Eukaryotic cells have higher amounts of nucleic acid than bacteria, and as a result a nuclease should be included to reduce the viscosity of the cell lysate.

2. EQUIPMENT

- French Pressure Cell
- Refrigerated centrifuge
- Magnetic stir plate
- Magnetic stir bars
- Polycarbonate centrifuge tubes

3. MATERIALS

- Tris base
- Hydrochloric acid (HCl)
- Sodium chloride (NaCl)
- Glycerol
- EDTA
- Phenylmethylsulfonyl fluoride (PMSF)
- Benzonase (Sigma)

3.1. Solutions & buffers

Step 1 Lysis Buffer

Component	Final concentration	Stock	Amount
Tris-HCl, pH 8.0	25 mM	1M	2.5 ml
NaCl	300 mM	5M	6 ml
Glycerol	10%	100%	10 ml
EDTA	1 mM	500 mM	0.2 ml
PMSF	1 mM	100 mM	1 ml

Add water to 100 ml

4. PROTOCOL

4.1. Duration

Preparation	Variable
Protocol	1h

4.2. Preparation

Generate a pellet of cells expressing the protein of interest (Recombinant protein expression in baculovirus-infected insect cells or Single Cell Cloning of a Stable Mammalian Cell Line). The length of this step is variable, depending on the method chosen to express the protein.

Chill the French Pressure Cell and piston to 4 °C, about 2 h.

See Fig. 4.1 for the flowchart of the complete protocol.

5. STEP 1 RESUSPEND CELLS IN LYSIS BUFFER

5.1. Overview

Resuspend the cell pellet in a lysis buffer in which the protein of interest will be stable, soluble, and not aggregated.

5.2. Duration

30 min

1.1 Resuspend the cell pellet in 2–5 times its volume of Lysis Buffer (e.g., resuspend a 5-ml cell pellet in 10–25 ml of Lysis Buffer).

1.2 Add 1 µl of Benzonase per ml of cell pellet.

1.3 Stir cells on a magnetic stir plate at 4 °C until the solution is homogeneous and no cell clumps are visible.

5.3. Tip

The lysis buffer should be modified to ensure that the protein of interest is stable, soluble, and not aggregated (see Explanatory Chapter: Troubleshooting protein expression: what to do when the protein is not soluble).

5.4. Tip

Avoid vortexing the cell suspension or introducing bubbles throughout this protocol or the protein may become denatured.

5.5. Tip

DNase and RNase can be used in place of the Benzonase.

5.6. Tip

Protease Inhibitor cocktails can be used in place of the EDTA and PMSF.

5.7. Tip

EDTA should be omitted if the cell lysate is going to be used with metal affinity chromatography.

See Fig. 4.2 for the flowchart of Step 1.

6. STEP 2 LYSE CELLS USING A FRENCH PRESS**6.1. Overview**

Physical force disrupts the cells.

6.2. Duration

5 min

2.1 Load the resuspended cells into the prechilled French Pressure Cell.

2.2 Lyse the cells with one pass of the piston on the high setting, ~2500 psi.

2.3 Collect lysate and keep at 4 °C.

See Fig. 4.3 for the flowchart of Step 2.

7. STEP 3 CLARIFY THE CELL LYSATE**7.1. Overview**

In this step, you remove any insoluble and aggregated material from lysate.

7.2. Duration

30 min

3.1 Spin lysate in a centrifuge at 18 000×g at 4 °C for 30 min.

3.2 The supernatant should be used immediately in the next step of the purification protocol for the protein.

7.3. Tip

Storing or freezing the cell lysate can lead to protein aggregation and degradation.

7.4. Tip

Protein solubility can be analyzed by comparing the amount of protein in the lysate before centrifugation and the amount in the supernatant and pellet after centrifugation. If the amount of protein in the clarified lysate is low, the lysis buffer should be modified accordingly to increase the solubility of the protein.

See Fig. 4.4 for the flowchart of Step 3.

REFERENCES

Referenced Protocols in Methods Navigator

1. Recombinant protein expression in baculovirus-infected insect cells.
2. Single Cell Cloning of a Stable Mammalian Cell Line.
3. Explanatory Chapter: Troubleshooting protein expression: what to do when the protein is not soluble.

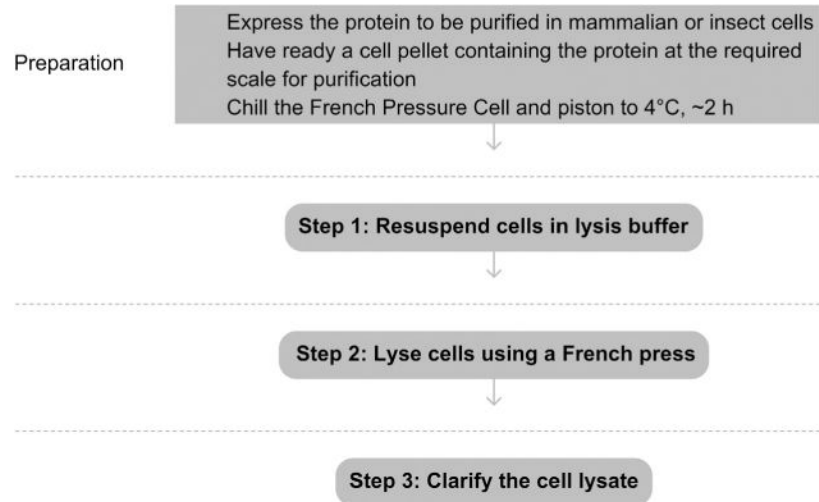


Figure 4.1.
Flowchart of the complete protocol, including preparation.

Step 1: Resuspend cells in lysis buffer

1.1 Resuspend cell pellet in 2-5 times its volume of Lysis Buffer



1.2 Add 1 μ l Benzonase per ml of cell pellet



1.3 Stir cells on a magnetic stir plate at 4°C until suspension is homogeneous

Figure 4.2.
Flowchart of Step 1.

Step 2: Lyse cells using a French press

2.1 Load resuspended cells into pre-chilled French press



2.2 Lyse cells with one pass of the piston on the high setting (~2,500 psi)



2.3 Collect lysate and keep at 4°C

Figure 4.3.
Flowchart of Step 2.

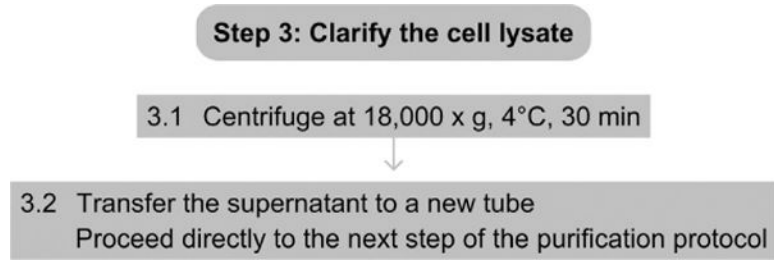


Figure 4.4.
Flowchart of Step 3.