

Preparation of clarified cell lysates for protein purification - Bacterial protein extraction reagent (B-PER)

Aim:

- Cell lysis and release of the protein of interest

Timeframe

- 30-35 minutes

Materials

- B-PER
- Nucleases: Benzonase (optional)
- Lysozyme (Optional)

Procedure

1. Pellet bacterial cells by centrifugation at $5000 \times g$ for 10 minutes.
2. Add 4mL of B-PER Reagent per 1g of cell pellet. Pipette the suspension up and down until it is homogeneous. Note: If using the B-PER II Reagent, 2mL of reagent per gram of cell pellet may be used to achieve a more concentrated protein solution.
3. Incubate 10-15 minutes at room temperature.
4. Centrifuge lysate at $15,000 \times g$ for 5 minutes to separate soluble proteins from the insoluble proteins.
5. Proceed with protein purification from soluble fraction.
6. Optional: (if not lysing add $2\mu\text{L}$ of lysozyme and $2\mu\text{L}$ of DNase I per 1mL of B-PER and add EDTA free protease inhibitors).