Cell Lysis using B-PER followed by SDS-Sampling and Bradford

Last updated: 2026-09-01

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Materials:

- B-PER reagent
- Bradford reagent (including BSA standard curve)
- Cell wash buffer (500mM NaCl, 20 mM sodium phosphate, pH=?)

This protocol is based on the assumptions:

- Cell culture aliquots (1mL) are already taken and stored
- Cell culture OD600 is known
- Pellet weight of the cell culture is known

Steps:

1. Pellet bacterial cells by centrifugation at 5000 × g for 10 minutes.
2. Add the B-PER II Reagent, 2 mL of reagent per gram of cell pellet.
3. Incubate 10-15 minutes at room temperature.
4. Add enough Cell Wash Buffer for the ‘OD600’ of the mixture to reach 20 (use table Cultures for tag assessment.xlsx for help).
    a. Note - based on rule of thumb (protein in 1 mL culture (mg) ≈ OD600 × 0.25), this mixture will have a protein concentration of around 5 mg/mL
    b. Note - do NOT use potassium phosphate buffer, as its not compatible with SDS downstream applications)
5. Take an aliquot for SDS preparation (e.g. 15-20 µL) (if total cell protein fraction is needed).
6. Take an aliquot for Bradford assay to determine total cell protein concentration (e.g. 40 µL).
7. Centrifuge lysate at 15,000 × g for 5 minutes to separate soluble proteins from the insoluble proteins (sample should look clear, not cloudy).
8. Take an aliquot for SDS preparation (e.g. 15-20 µL) (if soluble protein fraction is needed).
9. Take an aliquot for Bradford assay to determine soluble protein concentration (e.g. 40 µL).
10. Prepare a series dilution of aliquots taken for Bradford for it to fit into the calibration curve range (e.g. 20x, 50x, 100x dilutions?).