Protocol for cell lysis using NEBExpress *E. coli* Lysis Reagent (NEB #P8116)

Overview

1. If monitoring cell density by OD_{600}, record final OD readings prior to harvesting.

2. Harvest cells by centrifugation at 16,000 x g for 10 minutes. For larger volumes, centrifuge for ≥30 minutes especially if at ≤10,000 x g. Discard the medium and, if necessary, weigh the wet cell pellet.

3. Store the pellet at -20°C or -80°C or process immediately.

4. Resuspend the cell pellet in NEBExpress *E. coli* Lysis Reagent by pipetting or vortexing briefly until the suspension is homogenous:

    - Use 0.025 - 0.075 mL of NEBExpress *E. coli* Lysis Reagent for every 1 UOD_{600} harvested. To calculate the UOD_{600}, multiply the volume harvested by the OD_{600} reading.
    For example, a 5 mL culture harvested at OD_{600} 1 gives 5 mL x 1.0 = 5 UOD_{600}. In this example, 0.125 – 0.375 mL Lysis Reagent is required to lyse efficiently.

    - If harvested cells are weighed, use 5 mL of NEBExpress *E. coli* Lysis Reagent per 1 gram of cells.

5. Incubate the resuspended cells at room temperature for 10 - 20 min with gentle shaking, gentle rotation, or swirling. Lysis is usually visible with a clearance of the suspension.

6. Centrifuge the lysate at 16,000 x g for 10 min at 4°C to pellet the insoluble material and cell debris (30 min or longer for large volumes and lower speed).

7. Carefully transfer the supernatant into a sterile container for analysis or purification. This soluble fraction can be stored at 4°C for a few hours or -20°C or -80°C for longer term storage.

8. If needed, resuspend the insoluble pellet in 50 mM Tris-HCl pH 7.5 or any desired buffer for analysis or purification of inclusion bodies.

Notes:

- *A sample of the soluble fraction or resuspended insoluble fraction can be analyzed by SDS-PAGE. For 100 µl lysate with 1 UOD_{600} of cells , mix 20 µL of the fraction with 10 µL of 3x SDS Blue Loading buffer (NEB #B7703S). After heating 2 min at 95°C, the denatured proteins can be analyzed on SDS-PAGE by loading 10 to 20 µL per well or be stored at -20°C until analysis.*
- *The Lysis Reagent can efficiently lyse E. coli using up to 10 µL for 1 UOD_{600} of cell pelleted, which is is equivalent to 30 mL of Lysis Reagent for 1 L of cells at OD_{600} ~3. Beyond this ratio, the cell suspension will be too dense to lyse efficiently.*
- *Protease inhibitors can be added if needed.*
- *NEBExpress® T4 Lysozyme (NEB #P8115) can be added to improve protein extraction efficiency. Add 1 µL of NEBExpress® T4 Lysozyme per 1 mL of lysate. The lysate may become viscous and require addition of 200-2000 U/ml Micrococcal Nuclease (NEB #M0247S) or 10-100 U/ml DNase I (NEB #M0303) in the presence of 1mM $CaCl_2$.*