Montgomery County Community College
340 DeKalb Pike
Blue Bell, PA, 19422

SOP: Preparation of Bacterial Cell Lysate using B-PER Reagent

Approvals:

Preparer: Robin M. Zuck Date: 8MAR2019
Reviewer: Hetal Doshi Date: 10APR2019
Reviewer: Dr. Margaret Bryans Date: 28MAY2019

1. **Purpose:**
   1.1. This document details a method for preparing clarified bacterial cell lysates using the B-PER reagent, (Bacterial Protein Extraction Reagent by ThermoScientific product #78248).

2. **Scope:**
   This method applies to the preparation of small volumes of clarified bacterial cell lysates suitable for further purification of expressed recombinant proteins. The lysates can be prepared from frozen pelleted cells or freshly cultured cells. Mechanical disruption of the cells is not required.
   This procedure pertains to the preparation of 1 to 1.5 ml of lysate to be used for chromatography purification.
   This procedure can be used for larger volumes by maintaining approximately a 1:10 (w/v) ratio of cell pellet to B-PER reagent.

3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. **References:**
   4.1. User Guide: B-PER Bacterial Protein Extraction Reagent (www.thermofisher.com).
5. **Precautions:**
   5.1. Routine care should be exercised in the handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.
   5.2. Gloves, protective eyewear and a lab coat should be worn.
6. **Materials:**
   6.1. Balance
   6.2. Table top centrifuge
   6.3. Vortex
   6.4. Shaker
   6.5. Ice bucket and ice
   6.6. B-PER reagent, (ThermoFisher catalog number 78243)
   6.7. 1.5 ml Microfuge tubes with caps
   6.8. 0.45µm syringe filter and 1ml syringe
   6.9. Frozen cell pellet or pelleted bacterial cells
7. **Procedure:**
   7.1. Thaw frozen cell pellet on ice or place freshly pelleted cells on ice.

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7.2. Weigh an empty microfuge tube and record the weight.
7.3. Clean a narrow spatula with 70% Ethanol. Using the spatula, transfer approximately 100 mg of the cell pellet to the pre-weighed micro tube. Weigh the tube and contents. Determine the weight of cell pellet transferred to the tube.
Note: 100mg of pellet will occupy a volume approximately equal to the 0.1volume mark on the micro tube.
7.4. Add 1,000ul of B-PER reagent to the tube containing the cells.
7.4.1. If desired protease inhibitors can be added to the reagent.
7.5. Completely resuspend the pellet by pipetting up and down.
7.6. Vortex the suspension for 10 seconds.
7.7. Gently mix for 10 minutes at room temperature by manually inverting the tube or using a wrist shaker.
7.8. Centrifuge the lysate for 10 minutes at 15,000xg.
7.9. Transfer the supernatant to a fresh microtube and place on ice.
7.10. If the sample is to be purified by chromatography just prior to use sterile filter the lysate using a low protein binding 0.45µm syringe top filter and syringe

8. History:

Name	Date	Amendment