SOP: PP007.4
Modified 5-06-15

Preparation of Whole Cell Lysate

Materials and Reagents:

1. 100 g γ-irradiated *M. tuberculosis* whole cells
2. PBS (pH 7.4) with 1 mM EDTA
3. Complete, EDTA-free protease inhibitor tablets (Roche, 11 873 580 001)
4. DNase: 1 mg/ml stock (stored at -20ºC)
5. RNase: 1 mg/ml stock (stored at -20ºC)
6. 3500 MWCO dialysis membrane
7. Ammonium bicarbonate
8. Ice bucket
9. Ice
10. French Press
11. French Press cell
12. 225 ml conical falcon tube
13. Vortexer
14. Table top centrifuge
15. Plastic pipets (25 and 50 ml)
16. Graduated cylinders (100 and 250 ml)
17. Beaker (200 ml)
18. Dialysis tank (7 L)

Protocol:

1._____ Thaw 100 g of γ-irradiated *M. tuberculosis* cells. Thawing of the bacterial pellet should be performed at 4ºC (note 1).

2._____ Make breaking buffer by adding 1 stock of 30 µl DNase, 1 stock of 30 µl RNase, and one Complete, EDTA-free tablet to every 50 ml of PBS-EDTA buffer (note 2).

3._____ Add 1 ml of breaking buffer to every 2 g of thawed bacterial cells. (50 ml of breaking buffer to the 100 g thawed bacterial cells)

4._____ Create a homogeneous suspension of bacterial cells by vortexing for 30 sec and place on ice.

5._____ Place 40 to 45 ml of cell suspension in French Press cell (note 3).

6._____ Place French Press cell in French Press, collect lysate as it is forced out of the cell with a constant pressure of 1000 PSI as measured by the gauge on the French Press.

7._____ Place the lysate on ice.

8._____ Repeat Steps 5 to 7 until all of the cell suspension has passed through the French Press cell.

9._____ Repeat steps 5-8 five more times. Thus the total volume of cells should go through the French Press cell 6 times (note 4).

10._____ Add an equal volume (~ 150 ml) of PBS-EDTA buffer to the lysate and mix.

11._____ Centrifuge the lysate 3,000 rpm, 4°C in the table top centrifuge for 15 minutes to remove unbroken cells.

12._____ Collect the supernatant, this is the whole cell lysate. Unbroken cells (pellet) should be placed in a biohazard bag and autoclaved before discarding.

13._____ Prepare 3,500 MWCO dialysis tubing by rehydrating in endotoxin-free MilliQ H_20.

14._____ Prepare 7 L of dialysis buffer (10mM Ammonium bicarbonate) in a dialysis tank.

15._____ Add the whole cell lysate to the dialysis tubing. Close the dialysis tubing and place in the dialysis buffer.

16._____ Dialyze at 4ºC for 24 hours with three buffer changes.

17._____ Collect the protein solution from the dialysis tubing and rinse the dialysis tubing with a minimal volume (~2 ml of fresh 10 mM ammonium bicarbonate.

18._____ Remove a small aliquot (100 μl) for QC.

19._____ Estimate protein concentration by BCA (note 5).

20._____ Run 4 μg on a gel and silver stain (note 6).

21._____ Aliquot (default quantity is 10 mg) and store the whole cell lysate at -80°C

Notes:

1. This protocol deals with highly labile proteins and proteases that are found in *Mycobacterium tuberculosis* strains. It is important to keep the samples cool at all times, storing them for short periods of time at 4°C (or on ice) and for long periods of time at -80°C.
2. It is important to keep the buffer cool (in a refrigerator or on ice) after the addition of the protease inhibitor tablet, DNase, and RNase.
3. See SOP SP027 for use of the French press.
4. At this point the efficiency of cell lysis should be checked by acid fast staining and microscopy (see SOP SP035). At least 90% of the cells should be lysed.
5. The whole cell lysate will not be completely in solution, so be sure that it is a uniform suspension before performing the BCA and all other QC procedures. See SP003 for BCA.
6. See SOP SP007 for running gels and SP012 for silver staining.