

SOP: PP007.2
Modified 1-29-09

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 -20°C)
5. RNase: 1 mg/ml stock (stored at -20°C)
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 4°C (note 1).
2. ____ Make breaking buffer by adding 30 μ l DNase stock, 30 μ l RNase stock, and one Complete, EDTA-free tablet to 50 ml of PBS-EDTA buffer (note 2).
3. ____ Add 50 ml of breaking buffer to the 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. ____ To the lysate add an equal vol (~ 150 ml) of PBS-EDTA buffer and mix.
11. ____ Centrifuge the lysate 3,000 rpm, 4°C in the table top centrifuge for 15 minutes.
12. ____ Collect the supernatant, this is the whole cell lysate.

13. _____ Dialyze the whole cell lysate against 10mM ammonium bicarbonate for 24 hours at 4°C with three buffer changes.
14. _____ Estimate protein concentration by BCA (note 5).
15. _____ Remove a small aliquot (100 µl) for QC.
16. _____ Run 4 µg on a gel and silver stain (note 6).
17. _____ 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.
6. See SOP SP007 for running gels and SP012 for silver staining.