SOP: PP039.1
Modified: 11/25/2014 by MCL

LAM Removal from Hydrophobic/Mixed Subcellular Fractions
(WCL, CW, MEM) (note 1)

Materials and Reagents:

1. 50 mg subcellular fraction
2. 35 ml Teflon (oak ridge) tubes
3. Phosphate Buffered Saline
4. PBS Saturated Phenol (note 2)
5. Vortex Mixer
6. Lyophilizer
7. Rocker
8. Magnetic Stir Bar
9. Glass Pasteur Pipets and bulb
10. Sorvall Centrifuge
11. Dialysis tubing, 3500 MWCO
12. Bath Sonicator
13. Lyophilizer Flask
14. MilliQ Water
15. SDS-PAGE Supplies
16. Western Blot Supplies
17. 50 ml Falcon tubes

Protocol:

1._____ Obtain 50 mg of subcellular fraction and transfer to an Oak Ridge tube

2._____ Freeze dry by lyophilization. (see SOP:SP004)

3._____ Resuspend in 15 ml PBS.

4._____ Stir and vortex at room temperature until a good suspension is achieved.

5._____ While stirring, add 15 ml of PBS Saturated Phenol, 1ml at a time (note 2).

6._____ Rock at room temperature, in the hood, for 4 hours.

7._____ Centrifuge at 27,000xg, 25°C, for 1 hour.

8._____ Remove the aqueous (upper) layer without disturbing the interface. Note the volume removed. Save the aqueous layer.

9._____ To the phenol layer; add a volume of PBS equal to amount of volume removed in step 8.

10._____ Rock at room temperature, in the hood, for 1.5 to 2 hours.

11._____ Repeat steps 7 and 8.

12._____ Transfer the final phenol layer plus interface to 3500 MWCO dialysis tubing (do not include the pellet, if there is one). Do not fill the tubing more than half full. This layer is the subcellular fraction minus LAM

13._____ Transfer the saved aqueous layers to a separate 3500 MWCO dialysis tubing. (This layer includes the LAM and will be tested to confirm the presence of LAM and removal of proteins)

14._____ Place both in a dialysis tank and dialyze for 48-72 hours in running DI water. Gently knead the tubing occassionally, being sure to wear gloves, to break up chunks that form.

15._____ Transfer dialysis tubing to MilliQ water and dialyze at 4°C for at least 24 hours; continuing to knead.

16._____ Remove tubing from dialysis and transfer to individual 50 ml Falcon tubes. Be sure to rinse the phenol layer tubing well and not leave behind any material.

17._____ Sonicate and/or manually disrupt any large aggregates until a uniform suspension is achieved (note 3).

18._____ Vortex thoroughly, and perform a BCA on the phenol fraction (see SOP:SP003).

19._____ Run a gel on 4 µg of the phenol layer and ~5 µl of the aqueous layer of gel and western blot (developed against CS-35 (or other anti-LAM antibody) to confirm that there is no LAM in your fractions and that there is no protein in the aqueous layer (see SOP: SP011 and SP012).

Notes:

1. This protocol is specific for LAM removal from subcellular fractions containing hydrophobic or a mixture of hydrophilic and hydrophobic proteins, such as whole cell lysate, cell wall, and membrane. Fractions containing only hydrophilic proteins, such as cytosol and CFP, can use SOP: PP028. That protocol uses triton, which is less hazardous than using phenol (as is used here).
2. Use caution while working with phenol. Always handle phenol in a chemical fume hood, wearing proper PPE, and only use glass pipettes. Be sure that all centrifuge tubes used are compatible with phenol (the recommendation here is Teflon).
3. Some fractions will require a lot of sonication and will never fully go into solution, but a uniform suspension should be achieved before moving on.