SOP PP028.2
Modified: 11/25/2014 by MCL

LAM Removal from Hydrophilic Subcellular Fractions (CFP, CYT) (note 1)

Materials and Reagents: (note 2)
1. 20mg subcellular fraction, dry (note 3)
2. 32% Triton X-114 (Sigma, Catalog # X-114; note 4)
3. PBS, pH 7.4 (Gibco, Catalog # 10010-023)
4. Cold Absolute Ethanol (note 5)
5. 15ml Falcon Disposable Centrifuge Tubes (BD Falcon, Catalog #352097)
6. Teflon Oakridge Centrifuge Tubes (Nalgene, Catalog #3114-0050)
7. Sorvall Centrifuge with SS34 rotor
8. 37°C Incubator (or water bath)
9. Benchtop centrifuge

Protocol:

1._____ Resuspend dried sample in 10ml 4% triton X-114 (1.25ml 32% triton, 8.75ml PBS) and transfer to a 15ml falcon centrifuge tube.

2._____ Parafilm tube and rock at 4°C overnight.

3._____ Place tube in 37°C incubator (or 37°C water bath) until biphase is visible (approximately 30-60 minutes).

4._____ While sample is incubating, warm the benchtop centrifuge and rotor to 25°C.

5._____ Centrifuge sample at 3500rpm, 25°C, for 30 minutes.

6._____ Pipet aqueous (upper) layer into a clean falcon centrifuge tube, taking care not to pull off any of the triton layer with it.

7._____ Add 1.25 ml 32% triton to the aqueous layer, then fill the tube to 10ml total volume with PBS.

8._____ Parafilm tube and rock at 4°C for at least 2 hours (can go overnight again if needed).

9._____ Repeat incubation and centrifugation as in steps 3-5.

10._____ Pipet aqueous layer into two clean oakridge centrifuge tubes (note 6).

11._____ Fill the tubes with 30ml cold ethanol (this should be approximately 9ml ethanol per 1ml aqueous material).

12._____ Place in -20°C freezer overnight.

13._____ Centrifuge at 27,000xg, 4°C, for 30 minutes.

14._____ Decant supernatant into a waste container.

15._____ Add 35ml cold ethanol to each pellet.

16._____ Use a spatula to dislodge the pellet from the side of the centrifuge tube. This will ensure that the pellet gets thoroughly washed.

17._____ Repeat centrifugation as in step 13.

18._____ Decant supernatant into waste container (note 7).

19._____ Leave tubes open in a chemical fume hood until the pellet dries.

20._____ Resuspend dried pellet in your buffer of choice (generally 10 mM ammonium bicarbonate), quantitate by BCA, and run 4 µg on gel and western blot developed against CS-35 (or other anti-LAM antibody) to confirm the absence of LAM.

Notes:

1. This protocol is specific for LAM removal from hydrophilic subcellular fraction such as cytosol and CFP. Fractions such as whole cell lysate, cell wall, and membrane containing hydrophobic, or a mixture of hydrophilic and hydrophobic proteins would have a significant loss of hydrophobic proteins in the triton layer using this protocol and should instead use SOP: PP039.
2. The catalog numbers given are for materials used in the Dobos lab. Other materials can be used based on your laboratories equipment and resources.
3. More or less starting material can be used based on need. Be sure to adjust volumes to accommodate. Recovery from this protocol is approximately 40%.
4. See SOP R001 for preparation of 32% Triton
5. The ethanol should be chilled in a -20°C freezer for at least 24 hours before use.
6. At this point, be sure that the tubes used are compatible with ethanol and can accommodate a ten fold increase in volume. If the tubes are small, the aqueous material can be split into several tubes.
7. Often, after the second spin, the pellet does not adhere to the tube very well. It is important that you do not lose the pellet when you decant the ethanol. If necessary, you can leave a small amount of ethanol behind, rather than lose part of the pellet.