

## SOP PP028

### CFP Minus LAM

#### Materials and Reagents: (Note 1)

1. 20mg CFP, dry (Note 2)
2. 32% Triton X-114 (Sigma, Catalog # X-114; Note 3)
3. PBS, pH 7.4 (Gibco, Catalog # 10010-023)
4. Cold Acetone (Note 4)
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 biphasic 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 5).
11. \_\_\_\_\_ Fill the tubes with 30ml cold acetone (this should be approximately 9ml acetone 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 (Note 6).
15. \_\_\_\_\_ Add 35ml cold acetone 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.

**Notes:**

1. The catalog numbers given are for materials used in the Belisle lab. Other materials can be used base on your laboratories equipment and resources.
2. This protocol can be used to remove LAM from other subcellular fractions as well. Also, more or less starting material can be used base on need. Be sure to adjust volumes to accommodate. Recovery from this protocol is approximately 40%.
3. See SOP R001 for preparation of 32% Triton
4. The acetone should be chilled in a -20°C freezer for at least 24 hours before use.
5. At this point, be sure that the tubes used are compatible with acetone and can accommodate a ten fold increase in volume. If the tubes are small, the aqueous material can be split into several tubes.
6. Acetone is considered hazardous waste. Be sure that it is disposed of properly.
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 acetone. If necessary, you can leave a small amount of acetone behind, rather than lose part of the pellet.