SOP: PP011.2
Updated 4/7/17

Preparation of mAGP

Materials and Reagents:

1. Cell wall from *M. tuberculosis*
2. Phosphate buffered saline (PBS), sterile (Gibco 10010-023)
3. Sodium dodecylsulfate (SDS)/Lauryl sulfate (Sigma L-3771)
4. Water, HPLC-grade (VWR BJ365-4)
5. Acetone, HPLC-grade (VWR BJ010-1)
6. Proteinase K (Roche 1000144), stock solution of 10 mg/ml
7. Magnetic stir bar, small
8. Magnetic stir/heat plate
9. Magnetic stir bar retriever
10. Sorvall centrifuge
11. Sorvall SS-34 centrifuge rotor
12. Savant SpeedVac
13. Oakridge centrifuge tube, 50 ml, teflon, sterile
14. Warm room or reach-in incubator, 37°C
15. Beaker, 250 ml
16. Thermometer
17. Chemical fume hood
18. Glass tubes with PTFE-lined lid, 13 x 100 mm
19. Mettler-Toledo balance

Protocol:

1._____ Transfer *M. tuberculosis* cell wall to a sterile 50 ml Oakridge tube.

2._____ Add 30 ml of 2% SDS solution for each pellet and a small magnetic stir bar (note 1).

3._____ Cap tube and place on magnetic stir plate at room temperature for 30 minutes.

4._____ Remove the stir bar and centrifuge at 27,000 x g, 25°C for 10 minutes.

5._____ Decant the supernatant.

6._____ Repeat steps 2 to 5 twice more.

7._____ Add 26 ml of 2% SDS solution, 4 ml of Proteinase K stock solution and a small magnetic stir bar.

8._____ Cap tube and place on magnetic stir plate at room temperature for 30 minutes.

9._____ Remove the stir bar and centrifuge at 27,000 x g, 25°C for 10 minutes.

10._____ Decant the supernatant.

11._____ Add 30 ml of 2% SDS solution and a small magnetic stir bar.

12._____ Cap tube and place on magnetic heat/stir plate at 90°C for one hour (note 2).

13._____ Remove the stir bar and centrifuge at 27,000 x g, 25°C for 10 minutes.

14._____ Decant the supernatant.

15._____ Repeat steps 11 to 14 twice more.

16._____ Add 30 ml of water per pellet and a small magnetic stir bar.

17._____ Cap tube and place on magnetic stir plate at room temperature for 30 minutes.

18._____ Transfer sample, but not the stir bar, to a sterile 50 ml Teflon Oakridge tube.

19._____ Centrifuge at 27,000 x g, 25°C for 10 minutes.

20._____ Decant the supernatant. Repeat water wash (steps 16-19) twice more.

21._____ Add enough 40% acetone to each Oakridge tube to make nearly full.

22._____ Cap tubes and place on magnetic stir plate at room temperature for 10 minutes.

23._____ Remove the stir bar and centrifuge at 27,000 x g, 25°C for 10 minutes.

24._____ Decant and repeat acetone wash (steps 21-23) with 80%, then again with 100% acetone.

25._____ Decant acetone and place in chemical fume hood and allow to dry completely (note 3).

26._____ Transfer mAGP to tared 13 x 100 mm tubes and dry further on Savant.

27._____ Determine weight of mAGP in each tube.

28._____ Resuspend 1.0-2.0 mg mAGP from one of the tubes in 50% acetone in PBS to 2.0 mg/ml. Waterbath sonicate to ensure good resuspension.

29._____ Dilute mAGP further to make 1.0, 0.5, and 0.2 mg/ml suspensions also in 50% acetone/PBS.

30._____ Perform SDS assay to ensure sample is below 0.001% SDS (note 4).

31._____ Perform BCA assay to ensure protein is below 0.1 mg/ml (note 5).

32._____ Resuspend known quantity of mAGP to 10 mg/ml in 50% acetone/PBS to fulfill required number of 5 mg aliquots (note 6).

Notes:

1. The 2% SDS solution is made with sterile PBS. Use 2 g of SDS per 100 ml of PBS. If more than one pellet of cell wall is used, thaw and collect into small bottle, and add an equal volume of 2% SDS solution.
2. Fill a 250 ml beaker with water and heat to 90°C on a heat/stir plate; monitor temperature of water with a thermometer, and add water as needed.
3. Do not dry sample on air bath, as the sample will be blown away with the acetone. Before placing in chemical fume hood, decant as much acetone as possible without decanting the pellet.
4. See SP030 for SDS assay. Dilute SDS standards with 50% acetone/PBS.
5. See SP003. Test sample at 2, 1, 0.5 and 0.2 mg/ml. High protein content may skew the amino acid results for PG, which will be derived from mAGP.
6. Employ exhaustive water bath sonication, transfer 0.5 ml per 13 x 100 mm tube, and cover each tube with foil. Use N2 bath to blow off the acetone, poking needles through foil. This will prevent mAGP from escaping if it becomes too dry. Finish drying on Savant.

Reference:

Daffe, M., *et. al.* Journal of Biological Chemistry. 1990. 265:6734.