

SOP: PP011

Preparation of mAGP

Materials and Reagents:

1. Cell wall from *M. tuberculosis*
2. Phosphate buffered saline (PBS), sterile (Gibco 10010-023)
2. Sodium dodecylsulfate (SDS)/Lauryl sulfate (Sigma L-3771)
3. Water, HPLC-grade (VWR BJ365-4)
4. Acetone, HPLC-grade (VWR BJ010-1)
5. Proteinase K (Roche 1000144), stock solution of 10 mg/ml
6. Magnetic stir bar, small
7. Magnetic stir/heat plate
8. Magnetic stir bar retriever
9. Sorvall centrifuge
10. Sorvall SS-34 centrifuge rotor
11. Oakridge centrifuge tube, 50 ml, teflon, sterile
12. Warm room or reach-in incubator, 37°C
13. Beaker, 250 ml
14. Thermometer
15. Chemical fume hood
16. Glass tube with PTFE-lined lid, 16 x 100 mm
17. 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 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 xg, 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 xg, 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 xg, 25°C for 10 minutes.
14. ____ Decant the supernatant.
15. ____ Repeat steps 11 to 14 twice more.
16. ____ Add 30 ml of water 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 xg, 25°C for 10 minutes.
20. _____ Decant the supernatant.
21. _____ Add 5 ml of acetone and a small magnetic stir bar.
22. _____ Cap tube and place on magnetic stir plate at room temperature for 10 minutes.
23. _____ Remove the stir bar and centrifuge at 27,000 xg, 25°C for 10 minutes.
24. _____ Decant acetone and place in chemical fume hood and allow to dry completely (note 3).
25. _____ Transfer the purified mAGP to a new, pre-weighed 16 x 100 mm glass tube and weigh.

Notes:

1. The 2% SDS solution is made with sterile PBS. Use 2 g of SDS per 100 ml of PBS.
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.

Reference:

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