

SOP: PP012**Preparation of Arabinogalactan****Materials and Reagents:**

1. AGP (mAGP without mycolates), 50 to 150 mg
2. Sulfuric acid, 0.05M
3. Barium carbonate
4. Water, HPLC-grade (VWR BJ365-4)
5. Reagents for alditol acetates (note 6)
6. Glass tubes with PTFE-lids, 16 x 100 mm
7. Magnetic stir bar, small
8. Magnetic stir plate
9. Warm room or reach-in incubator, 37°C
10. Magnetic stir bar remover
11. Benchtop centrifuge
12. Pasteur pipet, glass
13. Pasteur pipet bulb, rubber
14. Savant speed-vac
15. Glass tubes with PTFE-lined lids, 13 x 100 mm
16. Glass capillary pipet, 10 μ l
17. Glass capillary pipetor, 10 μ l
18. Gas Chromatograph

Protocol:

- 1._____ Transfer AGP into a new 16 x 100 mm glass tube (note 1).
- 2._____ Add 5 ml of 0.05 M sulfuric acid and a small magnetic stir bar.
- 3._____ Cap tube and place on a magnetic stir plate at 37°C for four days.
- 4._____ Remove the small magnetic stir bar and centrifuge at 3,000 x g, 25°C for 15 minutes.
- 5._____ Transfer the supernatant to a new 16 x 100 mm tube (note 2).
- 6._____ Add a few grains of barium carbonate (note 3).
- 7._____ Cap tube and place at room temperature overnight.
- 8._____ Centrifuge at 3,000 x g, 25°C for 15 minutes.
- 9._____ Transfer the supernatant to a new, pre-weighed, 16 x 100 mm tube (note 4).
- 10._____ Completely dry on savant speed-vac (note 5).
- 11._____ Re-suspend dried material in 5 ml of HPLC-grade water.
- 12._____ Transfer two 2.5 μ l aliquots to two 13 x 100 mm glass tubes.
- 13._____ Completely dry on savant and prepare alditol-acetate derivatives (note 6).
- 14._____ Analyze derivatives by GC to ensure the purified AG contains only arabinose and galactose.
- 15._____ Make 1.0 mg aliquots based on the dry weight, dry on the savant, and store at -80°C.

Notes:

1. AGP is obtained from SOP PP014.
2. At this point an insoluble pellet will be remaining; this is peptidoglycan, and should be further processed according SOP PP013.

3. Barium carbonate neutralizes the weak acid, and only a small amount is required to accomplish this.
4. It is important to leave behind the residual salt pellet in the bottom of the tube.
5. See SOP SP005 for use of the savant.
6. See SOP SP022 for derivative preparation and SP045 for GC operation.

Reference:

Personal correspondance with Dr. Michael R. McNeil, Mycobacterial Research Laboratories, Colorado State University, Fort Collins CO and Dr. Phillip Draper, National Institute for Medical Research, Mill Hill, London United Kingdom.