SOP: PP012.2
Modified: 6/13/2022 by CM

Preparation of Arabinogalactan

Materials and Reagents:

1. mAGP, 50 to 150 mg
2. 0.5% KOH in Methanol
3. 0.05M sulfuric acid
4. Barium carbonate
5. Water, HPLC-grade
6. Methanol, HPLC-grade
7. Chloroform, HPLC-grade
8. Reagents for alditol acetates (note 6)
9. Glass tubes with PTFE-lids, 16 x 100 mm
10. Oakridge Teflon FEP tube
11. Magnetic stir bar, small
12. Magnetic stir plate
13. Warm room or reach-in incubator, 37°C
14. Magnetic stir bar remover
15. High Speed centrifuge
16. Benchtop centrifuge
17. Pasteur pipet, glass
18. Pasteur pipet bulb, rubber
19. Savant speed-vac
20. Glass tubes with PTFE-lined lids, 13 x 100 mm
21. Glass capillary pipet, 10 µl
22. Glass capillary pipetor, 10 µl
23. Gas Chromatograph

Protocol:

1._____ Transfer mAGP into a sterile Oakridge Teflon FEP tube (note 1, note 2).

2._____ Resuspend in 30 ml of 0.5% KOH in methanol.

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 27,000 x g for 20 min.

5._____ Discard the supernatant and resuspend the pellet in 30 ml of Methanol.

6._____ Centrifuge at 27,000 x g for 20 min

7._____ Discard the supernatant and resuspend the pellet in 20 ml of Chloroform.

8._____ Add a stir bar and mix well in a stir plate for 5-10 minutes at room temperature.

9._____ Add 10 ml of Methanol and centrifuge at 27,000 x g for 20 min

10._____ Discard the supernatant and resuspend the pellet in 30ml of methanol.

11._____ Repeat centrifugation at 27,000 x g for 20 min.

12._____ Discard the supernatant and dry the pellet in the nitrogen bath. (Note 3)

13._____ Transfer the AGP to a clean 16 x 100 mm glass tube.

14._____ Add 5 ml of 0.05M sulfuric acid

15._____ Stir with a magnetic stir bar at 37C for four days.

16._____ Centrifuge at 3,000 x g, 25°C for 15 minutes (note 4)

17._____ Transfer the supernatant to a new 16 x 100 mm tube

18._____ To neutralize the acid, add a few grains of barium carbonate to the supernatant (note 5).

19._____ Cap tube and place at room temperature overnight.

20._____ Centrifuge at 3,000 x g, 25°C for 15 minutes.

21._____ Transfer the supernatant to a new, pre-weighed, 16 x 100 mm tube (note 6).

22._____ Completely dry on savant speed-vac (note 7).

23._____ Re-suspend dried material in 2.5 ml of HPLC-grade water (note 8).

24._____ Transfer two 50 µl aliquots to two 13 x 100 mm glass tubes.

25._____ Completely dry on savant and prepare alditol-acetate derivatives (note 9).

26._____ Analyze derivatives by GC to ensure the purified AG contains only arabinose and galactose.

27._____ Make 1.0 mg aliquots based on the dry weight, dry on the savant, and store at -80°C.

Notes:

1. mAGP is obtained from SOP PP011.
2. This protocol is not used for purification of Mycolic acid Methyl Esthers (MAME). For purification of MAME, refer to SOP PP014. Similarly, DO NOT use AGP produced from SOP PP014 as this product will contain high amounts of TBAH and will likely not pass QC.
3. The pellet consists of pure AGP.
4. At this point an insoluble pellet will be remaining; this is peptidoglycan, and should be further processed according SOP PP013.
5. Barium carbonate neutralizes the weak acid, and only a small amount is required to accomplish this.
6. It is important to leave behind the residual salt pellet in the bottom of the tube.
7. See SOP SP005 for use of the savant.
8. Take 10ul of the resuspended AG and put on a pH strip. If pH is still 0-3, it is recommended to do another neutralization with sodium carbonate to avoid potential inhibition of the derivatization reaction when doing alditol acetates.
9. See SOP SP022 for derivative preparation and SP045 for GC operation.

Reference:

Grzegorzewicz A & Jackson M. Subfractionation and analysis of the cell envelope (lipo)polysaccharides of Mycobacterium tuberculosis. Methods Mol Biol. 2013; 966: 309–324. doi:10.1007/978-1-62703-245-2_19