

SOP: PP013**Preparation of Peptidoglycan****Materials and Reagents:**

1. AGP (mAGP without mycolates), 50 to 150 mg
2. Sulfuric acid, 0.05M
3. Water, HPLC-grade (VWR BJ365-4)
4. Reagents for alditol acetates (note 4)
5. Glass tubes with PTFE-lids, 16 x 100 mm
6. Magnetic stir bar, small
7. Magnetic stir plate
8. Warm room or reach-in incubator, 37°C
9. Magnetic stir bar remover
10. Benchtop centrifuge
11. Vortex
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 0.05M 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 5 ml of HPLC-grade water to the pellet.
7. ____ Cap tube and vortex vigorously.
8. ____ Centrifuge at 3,000 x g, 25°C for 15 minutes.
9. ____ Decant the supernatant into a new, preweighed 16 x 100 mm tube and completely dry on savant speed-vac (note 3).
10. ____ Re-suspend the dried material in 5 ml of HPLC-grade water.
11. ____ Transfer two 25 μ l aliquots to two new 13 x 100 mm glass tubes.
12. ____ Completely dry on savant and prepare alditol-acetate derivatives (note 4).
13. ____ Analyze derivatives by GC to ensure the purified PG does not contain arabinose and galactose (note 5).
14. ____ Make 0.25 mg aliquots based on the dry weight, dry and store at -80°C.

Notes:

1. AGP is obtained from SOP PP014.

2. At this point the supernatant is soluble arabinogalactan, and should be further purified using SOP PP012.
3. See SOP SP005 for use of savant.
4. See SP022 for derivative preparation and GC operation.
5. If the GC analysis shows contaminating arabinose and/or galactose, then the protocol needs to be repeated, until the peptidoglycan is devoid of arabinogalactan.

Reference:

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