

**SOP: PP013.2**  
**Modified: 2/4/19 by DCH**

### **Preparation of Peptidoglycan**

#### **Materials and Reagents:**

1. AGP (mAGP without mycolates), 50 to 150 mg
2. Sulfuric acid, 0.25 M
3. Water, HPLC-grade (VWR BJ365-4)
4. Reagents for alditol acetates (note 4)
5. Methanol, ACS grade
6. Glass tubes with PTFE-lids, 13 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. Vortex
13. Pasteur pipet, glass
14. Pasteur pipet bulb, rubber
15. Savant speed-vac
16. Glass tubes with PTFE-lined lids, 13 x 100 mm
17. Glass capillary pipet, 10  $\mu$ l
18. Glass capillary pipet, 10  $\mu$ l
19. Gas Chromatograph
20. Glass vials, 2 ml

#### **Protocol:**

1. \_\_\_\_\_ Transfer AGP into a new 16 x 100 mm glass tube (note 1).
2. \_\_\_\_\_ Add 5 ml of **0.25 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 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. Decant supernatant.
9. \_\_\_\_\_ Repeat wash steps 6-8 2X, carefully decanting with a Pasteur pipet.
10. \_\_\_\_\_ Re-suspend the pelleted material in 5 ml of HPLC-grade water.
11. \_\_\_\_\_ Transfer three 50  $\mu$ l aliquots to two new 13 x 100 mm glass tubes.
12. \_\_\_\_\_ Completely dry on savant (note 3).
13. \_\_\_\_\_ Prepare alditol-acetate derivatives of diluted PG and neutral sugar standards (note 4).
14. \_\_\_\_\_ Analyze derivatives by GC to ensure the purified PG does not contain arabinose and galactose (note 5).
15. \_\_\_\_\_ Dry a separate 100  $\mu$ l aliquot of resuspended PG in small vial to obtain weight measurement.

16. \_\_\_\_\_ Resuspend PG in 50% MeOH at 1 mg/ml.
17. \_\_\_\_\_ Aliquot 50  $\mu$ l x3 for submission to UNMC for amino acid analysis (note 6).
18. \_\_\_\_\_ Make 0.5 mg aliquots based on the dry weight, dry and store at -20°C, or in the desiccator if being shipped within the next few days.

**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 SP045 GC operation.
5. Tape GC traces of neutral sugars and PG into notebook. If the analysis shows contaminating arabinose and/or galactose, then the protocol needs to be repeated, after centrifuging and obtaining a pellet again, until the peptidoglycan is devoid of arabinogalactan.
6. Submission form found at [www.unmc.edu/vcr/cores/vcr-cores/pscf/index.html](http://www.unmc.edu/vcr/cores/vcr-cores/pscf/index.html).

**Reference:**

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