

## Surface Polysaccharide Extraction and Quantification

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**[Abstract]** Gram-negative bacterial cells possess two membranes - the inner cytoplasmic membrane and the outer membrane. The two membranes are distinct in their composition; the inner membrane is composed of a phospholipid bilayer, whereas the outer membrane (OM) is composed of an asymmetrical bilayer, with the outer leaflet containing lipopolysaccharide (LPS) (Raetz and Whitfield, 2002). Surface polysaccharides, such as LPS O-antigen, or capsular polysaccharide, are often tightly associated with the OM (Whitfield, 2006). This tight association can be used to generate a rough quantification of surface polysaccharides of Gram-negative bacterial cells, as the OM can easily be dissociated from cells without associated cell lysis (Brimacombe *et al.*, 2013). The following method describes how to quickly extract and quantify OM-associated polysaccharides.

### **Materials and Reagents**

1. Culture of bacterial cells (This procedure works only for Gram-negative bacteria, for example *Escherichia coli*, *Pseudomonas aeruginosa*, or *Rhodobacter capsulatus*. The outer membrane, specifically LPS, is essential for this procedure to work)
2. 50 mM sodium chloride (NaCl) dissolved in deionized H<sub>2</sub>O
3. 50 mM ethylenediaminetetraacetic acid (EDTA) (EMD Millipore, catalog number: 324503)
4. Phenol (Fisher Scientific, catalog number: A92-100)
5. 93% sulfuric acid (Avantor Performance Materials, catalog number: 2900-10)
6. Carbohydrate stock solution (see Recipes)

### **Equipment**

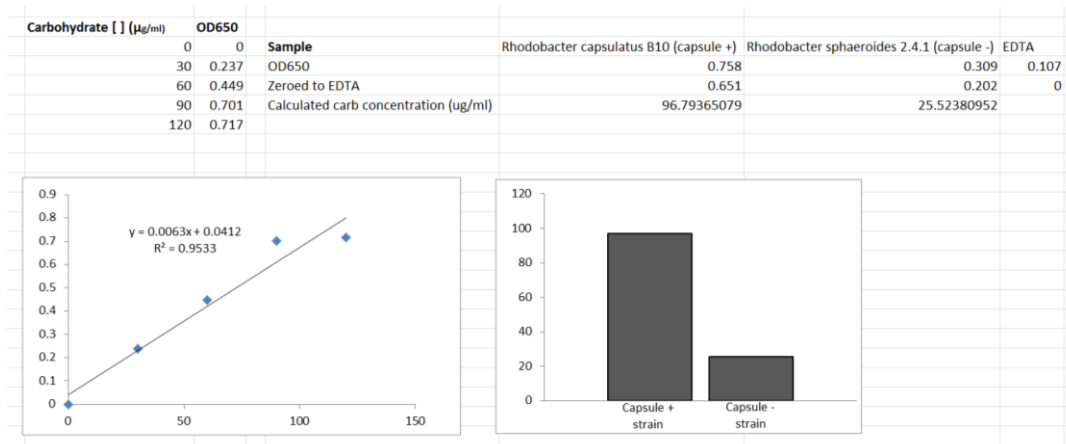
1. Microcentrifuge
2. Microfuge tubes (ESBE, catalog number: ESB-ES00507C)
3. Spectrophotometer
4. Cuvettes
5. Glass test tubes
6. Glass pipettes

## Procedure

- A. Extraction of surface polysaccharides from Gram-negative bacteria
1. Grow bacteria to desired growth phase (generally stationary phase) in desired growth medium.  
*Note: Growth medium may affect surface polysaccharide levels, so the same media should be used for all experiments if possible.*
  2. Measure OD<sub>650</sub> of culture; dilute to < 1 OD if necessary to get an accurate measurement.
  3. Normalize cultures to OD<sub>650</sub> of 2.0 (or to maximum OD that bacterial culture will grow to if it is less than 2.0).
  4. Harvest 1 ml of each normalized culture by centrifugation at 14,500 x g for 5 minutes in a microcentrifuge.
  5. Carefully remove supernatant with a pipette, discard tip.
  6. Wash cells by re-suspending in 1 ml of 50 mM NaCl, pellet by centrifugation at 14, 500 x g for 5 minutes, remove supernatant.
  7. Repeat step A6 four additional times (5 total washes).
  8. Re-suspend cells in 1 ml of 50 mM EDTA, and incubate at 37 °C for 60 minutes (EDTA causes LPS to dissociate, thus releasing the OM from cells).
  9. Pellet cells by centrifugation at 14,500 x g for 5 minutes, carefully remove supernatant and transfer to fresh microfuge tube (supernatant contains all surface polysaccharides, including LPS, capsule *etc.*).
- B. Quantification of surface polysaccharides
1. Prepare carbohydrate standards by diluting carbohydrate stock solution into 1 ml aliquots of: 0, 30, 60, 90, and 120 µg/ml of carbohydrate (*e.g.* 970 µl of dH<sub>2</sub>O + 30 µl of 1 mg/ml stock solution to generate a 30 µg/ml standard).
  2. Prepare clean, acid washed glass test tubes (for a suggested protocol, see Reference 4). Pipette 200 µl of standards, a 200 µl control of 50 mM EDTA, and all test samples into separate tubes.
  3. Move to fume hood.
  4. Add 200 µl of 5% phenol to all tubes, mix well by shaking.
  5. Add 1 ml of 93% sulfuric acid; mix well by swirling (use caution).
  6. Allow colour to develop for 10 minutes at room temperature (reaction should turn yellow; intensity depends on carbohydrate concentration). Additional mixing by gentle swirling every 2-3 minutes may help reaction proceed faster.
  7. Measure OD<sub>490</sub> of all reactions in a spectrophotometer; concentration of carbohydrates can then be calculated from the standard curve.

Note: If necessary, dilute reactions in dH<sub>2</sub>O to get accurate spectrophotometer readings.

### Representative Data



### Recipes

1. Carbohydrate stock solution  
50:50 mixture of 0.5 mg/ml each of sucrose and fructose  
Final concentration of 1 mg/ml carbohydrate (molecular biology grade recommended)

### Acknowledgments

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### References

1. Brimacombe, C. A., Stevens, A., Jun, D., Mercer, R., Lang, A. S. and Beatty, J. T. (2013). [Quorum-sensing regulation of a capsular polysaccharide receptor for the \*Rhodobacter capsulatus\* gene transfer agent \(RcGTA\)](#). *Mol Microbiol* 87(4): 802-817.
2. [http://openwetware.org/images/9/9e/GLASSWARE\\_CLEANING\\_PROCEDURES.pdf](http://openwetware.org/images/9/9e/GLASSWARE_CLEANING_PROCEDURES.pdf)
3. Raetz, C. R. and Whitfield, C. (2002). [Lipopolysaccharide endotoxins](#). *Annu Rev Biochem* 71: 635-700.
4. Whitfield, C. (2006). [Biosynthesis and assembly of capsular polysaccharides in \*Escherichia coli\*](#). *Annu Rev Biochem* 75: 39-68.