Colorimetric Carbohydrate Assay for Corals

Sample Preparation:

This protocol is designed to work with tissue slurry that has been processed by airbrushing and after remove symbiont cells and homoginization. Please see other protocols for these procedures.

Reagents:

1. Concentrated Sulphuric Acid (95% certified ACS grade)
2. Phenol (certified ACS)
3. MilliQ Water
4. Glucose (L-(−)-)

Assay

1. Pull desired coral slurries from the -80 freezer to thaw at room temperature
2. Collect test tubes and label (*10 for the standard + the number of samples running*)
3. Make the standards as shown below (*and printed in lab notebook – check them off as you add them*)
4. Vortex and spin down for 2 minutes, then add 100 µL of coral slurry and 900 µL milliQ water to pre-labelled test tube for all samples
5. Set up a room temperature water bath in the hood with test tube rack (*DI water in a plastic bin is fine*)
6. Add 25 µL of phenol to first sample (*in the refrigerator - use the small bottle and do not pull directly from the stock*)
7. Vortex (*in the hood*) for 3 seconds
8. Immediately add 2.5 mL sulphuric acid to the sample (*again, take from the aliquot and not the stock bottle*)
9. Incubate the sample at room temperature for 1 minute
10. Transfer sample to water bath
11. Repeat steps 6-10 for all tubes
12. When the last sample is placed in the water bath, incubate all samples for 30 minutes
13. Pipette 200 µL of all standards and samples into the bottom of the wells in a 96-well plate
14. Go to Caudill Lab 208 to read on the plate reader using the "Colleen and JB Carb" protocol (*absorbance 485 nm*)

Coral Total Carbohydrate Calculation*:

1. Create standard curve with known standard concentrations and absorbance values ($y = mx + b$)
2. Using the resulting equation, convert sample absorbance to concentrations (mg/mL)
3. Multiply sample concentration (mg/mL) by total slurry volume (mL) and dilution factor (1000/v of sample, usually 100 µL), then divide by surface area (cm^2) for resulting units: mg/cm^2

** Can use associated Rmarkdown script to calculate concentrations per sample well (Carb Calculation Script.Rmd)*

DOI: dx.doi.org/10.17504/protocols.io.bvb9n2r6 *GitHub: seabove7/protocols/ CarbohydrateAssay_96wellplate*