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Cyanobacteria Total Lipid Extraction from Polycarbonate Filters

Forked from Cyanobacteria Total Lipid Extraction

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ABSTRACT

This protocol is designed/used for extraction of total cellular lipids from cyanobacteria samples (either lab cultures or field samples) collected on polycarbonate filters for use in lipid analysis and quantification *via* mass spectrometry.

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) or Robbie M. Martin (rmarti49@vols.utk.edu) for additional information regarding this protocol.

Modified from Guan, X. L., Riezman, I., Wenk, M. R., & Riezman, H. (2010). Yeast lipid analysis and quantification by mass spectrometry. *Methods in Enzymology*, *470*, 369-391.

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1 Prepare the three separate solutions needed for this extraction protocol as follows:

- lipid extraction solvent: a 15 : 15 : 5 : 1 : 0.18 ratio by volume of 95% ethanol, water, diethyl ether, pyridine, and 4.2 N ammonium hydroxide, respectively.
- water-saturated butanol: a 1:1 ratio of butanol and Milli-Q water
- purified lab water: (Milli-Q water)

2 Unfold polycarbonate filter and place into a 2-mL centrifuge tube with cell side of filter facing outwards.

Note: Appropriate volume of lab culture or field samples to filter and extract depends on cell concentration. As a guideline, we have been successful filtering 10-25 mL of lab cultures of *Microcystis aeruginosa* and ~50 mL of either raw lake water or mesocosm samples.

3 Add 1 mL of extraction solvent, ~100 µL of glass beads, and vortex ~5 s.

4 Incubate sample in 60 °C water bath for 20 min.

5 Centrifuge sample at 10,000 x g for 10 min.

6 Remove supernatant and place into a 1-dram glass vial (dram vial #1). The first two extractions from a sample will be placed in this vial (#1).

7 Repeat steps 3-6, except DO NOT ADD more glass beads.

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8 Dry the collected supernatant in dram vial #1 under a stream of nitrogen.

9 Re-suspend dried sample in 300 µL of water-saturated butanol and 150 µL of Milli-Q water.

10 Vortex and transfer to a 2-mL centrifuge tube.

11 Centrifuge at 10,000 x g for 2 min.

12 Remove top butanol phase and place into a NEW 1-dram glass vial (dram vial #2).

13 Wash original dram vial (#1) with 300 µL saturated butanol and transfer to residual aqueous phase in 2-mL centrifuge tube from step 10. Vortex.

14 Centrifuge at 10,000 x g for 2 min. Remove top butanol phase and place into dram vial #2.

15 Dry the collected butanol phase in dram vial #2 under a stream of nitrogen.

16 Re-suspend dried sample in 300 µL of 9:1 methanol:chloroform.

17 The samples are now ready for analysis *via* LC/MS.

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