Cyanobacteria Total Lipid Extraction

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Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) or Maddie Denney (mdenney5@vols.utk.edu) for additional information regarding this protocol.

Modified from Guan, Riezman, Wenk & Riezman, 2010

Please note that there are two versions of this protocol. Use the one that corresponds to your sample.

Citation: Dr. Steven Wilhelm, Maddie Denney Cyanobacteria Total Lipid Extraction. **protocols.io** dx.doi.org/10.17504/protocols.io.ibkcakw
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Protocol

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 1.

Prepare lipid extraction solvent (step 2) with a 1:1 ratio of butanol and lab purified water (water-saturated butanol, and lab purified water

REAGENTS
Butanol 71-36-3 by Fischer Scientific

ANNOTATIONS
Maddie Denney 16 Jun 2017

This is written incorrectly. Should state: "Prepare lipid extraction solvent (step 2), 1:1 ratio butanol and lab purified water (water-saturated butanol), and lab purified water."

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 2.

Extraction solvent is a 15:15:5:1:0.18 ratio by volume solvent composed of 95% ethanol, water, diethyl ether, pyridine, and 4.2 N ammonium hydroxide

REAGENTS

Ethanol BE-BDH1156 by P212121

Diethyl ether 60-29-7 by Fisher Scientific

Pyridine 110-86-1 by Fisher Scientific

Ammonium hydroxide 7664-41-7 by Fisher Scientific

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 3.

Grow cultures to log phase and collect 2 mL in 2 mL microfuge tube.

AMOUNT

2 ml Additional info:

NOTES

Alyssa Alsante 05 Jun 2017

If the sample is a liquid environmental sample, simply proceed without grow up peroid. A minimum of 500 µL of sample is needed.

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 4.

Optional step: Remove a small aliquot (100 µL) of culture and fix with 5% by volume glutaraldehyde for later cell counts for normalizing lipid amounts. Store at 20ºC.

REAGENTS

25% Glutaraldehyde by Contributed by users

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 5.

Pellet culture at 10,000 xg, 2 min

DURATION

00:02:00

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 6.

Remove supernatant

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 7.

Add 1 mL extraction solvent to resuspend pellet

AMOUNT

1 ml Additional info:

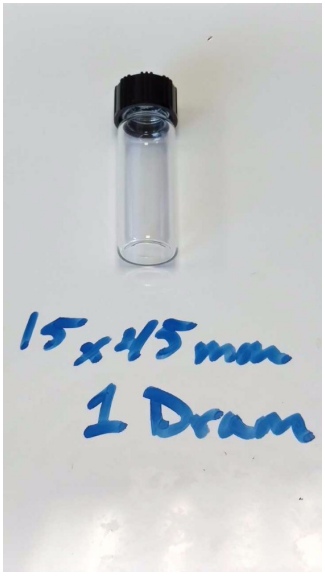
Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 8.

Add 100 µL of 150 µm glass beads in a 1 dram vial

AMOUNT
100 µl Additional info:

NOTES
Alyssa Alsante 13 Jun 2017



Version 1 - Lab Grown Cultures and Liquid Environmental Cultures
Step 9.

Vortex sample, 5 sec

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures
Step 10.

Incubate sample in a 60ºC water bath for 20 min

DURATION
00:02:00
Version 1 - Lab Grown Cultures and Liquid Environmental Cultures
Step 11.

Centrifuge sample at 10,000 xg for 10 min in a benchtop microcentrifuge

DURATION
00:10:00
Version 1 - Lab Grown Cultures and Liquid Environmental Cultures
Step 12.

Remove supernatant to a glass vial

NOTES
Alyssa Alsante 05 Jun 2017

All extractions for a single sample will be added to the same sample glass vial.

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 13.

Repeat steps 7 and 9-12; DO NOT add more glass beads

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 14.

Add 150 µL water to microfuge tube

AMOUNT

150 µl Additional info:

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 15.

Vigorously shake water-saturated butanol (1:1 ratio) to mix and add 300 µL to microfuge tube

AMOUNT

300 µl Additional info:

REAGENTS

Butanol 71-36-3 by Fischer Scientific

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 16.

Vortex sample, 5 sec

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 17.

Remove top butanol phase to sample glass vial

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 18.

Repeat steps 15-17

Version 1 - Lab Grown Cultures and Liquid Environmental Cultures

Step 19.

Either immediately dry sample under N_2 for LCMS or store overnight at -20ºC or longer term storage at -80ºC

Version 2 - Sterivex Filtered Environmental Sample

Step 20.

Prepare lipid extraction solvent as in steps 1-2 of version 1 above

Version 2 - Sterivex Filtered Environmental Sample

Step 21.

Use a Sterivex cutter to open the plastic unit. Using a sterile razor blade, cut filter off of unit and cut into small pieces. Place filter pieces in a 2 mL microfuge tube.

Version 2 - Sterivex Filtered Environmental Sample

Step 22.

Add 1 mL of extraction solvent to resuspend pellet

AMOUNT

1 ml Additional info:

Version 2 - Sterivex Filtered Environmental Sample

Step 23.

Add 100 µL of 150 µm glass beads

AMOUNT

100 µl Additional info:

Version 2 - Sterivex Filtered Environmental Sample

Step 24.

Vortex sample very vigorously, 5 sec -- do not want filter pieces to setlle at the bottom of microfuge tube

Version 2 - Sterivex Filtered Environmental Sample

Step 25.

Incubate sample in a 60ºC water bath for 20 min

DURATION

00:03:00

Version 2 - Sterivex Filtered Environmental Sample

Step 26.

Centrifuge sample at 10,000 xg, 10 min in a benchtop microcentrifuge

DURATION

00:10:00

Version 2 - Sterivex Filtered Environmental Sample

Step 27.

Remove supernatant to a glass vial

NOTES

Alyssa Alsante 05 Jun 2017

All extractions for a single sample will be added to the same glass vial.

Version 2 - Sterivex Filtered Environmental Sample

Step 28.

Repeat steps 22 and 24-27; DO NOT add more glass beads

Version 2 - Sterivex Filtered Environmental Sample

Step 29.

Add 150 µL water to microfuge tube

AMOUNT

150 µl Additional info:

Version 2 - Sterivex Filtered Environmental Sample

Step 30.

Vigorously shake water-saturated butanol to mix (1:1 ratio) and add 300 µL to microfuge tube

AMOUNT

300 µl Additional info:

Version 2 - Sterivex Filtered Environmental Sample

Step 31.

Vortex sample

Version 2 - Sterivex Filtered Environmental Sample

Step 32.

Remove top butanol phase to sample glass vial

Version 2 - Sterivex Filtered Environmental Sample

Step 33.

Repeat steps 30-32

Version 2 - Sterivex Filtered Environmental Sample

Step 34.

Either immediately dry sample under N_2 for LCMS or store overnight at -20ºC or longer term storage at -80ºC