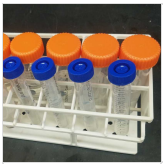

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Preparation of Carbon Sources for Possible Heterotrophic Feedstock for O. tauri

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1 *Works for me* This protocol is published without a DOI.

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SUBMIT TO PLOS ONE

ABSTRACT

Instructions for preparing stock carbon source solutions as experimental heterotrophic feedstocks for Ostreococcus tauri.

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KEYWORDS

Ostreococcus tauri, carbon source, heterotrophy, heterotrophic, feedstock, culture, media

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MATERIALS TEXT

- 50 mL Centrifuge Tubes, Sterile, Polypropylene, Globe Scientific (Fisher Scientific 22-010-066)
- 15 mL Centrifuge Tubes, Sterile, Polypropylene, Corning (Fisher Scientific 05-539-5)
- 22 um Syringe Filter, MCE Membrane, 23 mm diameter, Sterile Millex, MilliporeSigma (Fisher Scientific SLGSR33SB)
- 60 mL sterile single-use syringe, Fisherbrand (Fisher Scientific 14-955-461)
- Prepared ASW + K Medium (https://www.protocols.io/edit/keller-k-medium-in-artificial-sea-water-for-cultur-brv7m69n)
- Weigh paper, 4" x 4", Fisherbrand (Fisher Scientific 09-898-12B)
- Spatula, stainless (Fisher Scientific 14-375-20) or scoopula, stainless, 6" (Fisher Scientific 14-357Q)
- Balance, Analytical, Mettler-Toledo AB104-S
- Laminar Flow Hood, NuAire Class II, Type A2

SAFETY WARNINGS

Read safety data sheets for PPE and safety handling requirements of selected carbon sources.

DISCLAIMER:

This protocol is in the experimental design phase. It is not established that O. tauri can feed heterotrophically on any carbon source.

BEFORE STARTING

Sanitize walls and benchtop of laminar flow hood with 70% ethanol. Ensure fan is operational.

1 Determine amount of carbon source solution that is needed. Take into account both the amount needed, concentration needed, dilution factor, and the smallest amount that can be made while still accurately weighing solids and measuring liquids.

C Source	Stock C Source Vol Solid (g)	Stock C Source to Vol with ASW (mL)	Stock C source solution concentration (g/L)	Stock C source Sol'n Volume/Well (uL)	Pure ASW Volume/Well (uL)	Total Well Volume (uL)	Final concentration (g/L)	Molecular wt (g/mol)	Final conc (mM)
Sodium acetate 99%	0.45	30	15	60	240	300	3	82.03	36.57

Example table for calculating stock carbon source solution amount and components.

This example does not take into account the stock chemical purity when calculating concentration of g/L or Molarity of carbon source available for algae.

Stock chemicals come in anhydrous, monohydrate, and dihydrate forms. The hydration state of the stock chemical will affect calculated concentration (g/L and M) versus amount of carbon in the solution available to the microbes. Chemical concentrations of a mono or dihydrate form are not directly comparable to concentrations of anhydrous forms and will have less C available to the algae per g/L or per mole than anhydrous forms. Calculating carbon moles only may be the best way to directly compare solutions.

2 Weigh the carbon source chemical into an appropriately sized centrifuge tube.

Record the weight, the chemical name, the chemical manufacturer, and the lot number in your lab notebook.

Use fresh weigh paper or boats between carbon source chemicals. Use fresh spatula or scoopula or clean spatula or scoopula with 70% ethanol between each carbon source chemical.

3 In the sterilized laminar flow hood, add two thirds the required amount of prepared, sterile ASW + K culture medium.

Do not open sterile ASW culture media outside of the sterile laminar flow hood.

4 Mix solution by shaking vigorously until the carbon source chemical is completely dissolved. Most carbon sources are hydrophilic and highly soluble. If carbon source doesn't immediately go into solution, let rest for 10 minutes then shake vigorously again.

If after several rounds of resting and vigorous shaking, carbon source precipitate is still visible, investigate if

saturation levels of carbon source to volume have been exceeded.

Carbon sources are calculated on a weight by volume basis. If the chemical isn't fully dissolved before bringing the solution to the final volume, then the final solution concentration will not be accurate.

5 In the sterilized laminar flow hood, bring solution to final volume with prepared, sterile ASW + K culture medium. Record the final volume in your lab notebook.

For culture medium preparation, the markings on the side of the centrifuge tube are accurate enough for volume.

6 Mix thoroughly by shaking centrifuge tube vigorously until striations in the solution are not visible.

7 In the sterilized laminar flow hood, filter the carbon source solution into a new, sterile centrifuge tube using a sterile 0.2 um syringe filter.

8 Label the stock carbon source solution with the name of the solution, the concentration, initials, date, and the word sterile.

9 Store at **Room temperature** .

After sterilization, only open stock carbon source solutions in the sterilized laminar flow hood.

If solutions will be used multiple times, they could be autoclaved between uses to ensure that no biological contamination is introduced.