Algal Media Recipe from Cáceres Lab V.1

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Duffy Lab, EEB, University of Michigan

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This is a protocol to make media to grow algae in. In the Duffy Lab, this media is used to grow *Ankistrodesmus falactus*. This recipe comes from the Cáceres Lab.

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Algal Media Recipe from Cáceres Lab

Written by C. Caceres 2005

Reformatted by Isabella Oleksy 2013

Algal Nutrients

Stock solutions can be made up in 250mL, 500mL or 1L bottles. Use table to determine correct amount of nutrient to be added to desired amount of water. Use MilliQ water.

MICRONUTRIENTS

A	B	C	D	E
Name	Symbol	Add to 250mL	Add to 500mL	Add to 1L
(1) EDTA Disodium Salt	Na2EDTA	1.86g	3.72g	7.44g
(2) Boric acid	H3BO3	0.62g	1.24g	2.47g
(3) Maganese chloride	MnCl2 4H2O	0.346g	0.693g	1.385g
Cobalt chloride Sodium	CoCl2 6H2O	0.005g	0.010g	0.019g
molybdate	Na2MoO4 2H2O	0.003g	0.055g	0.010g
(4) Cupric sulfate	CuSO4 5H2O	0.0000465g**	0.000093g**	0.000186g**
Zinc sulfate	ZnSO4 7H2O	0.23g	0.46g	0.920g
(5) Ferric chloride	FeCl3 6H2O	0.27g	0.54g	1.081g

MACRONUTRIENTS

A	B	C	D	E
Name	Symbol	Add to 250mL	Add to 500mL	Add to 1L
(6) Potassium phosphate	K2HPO4	4.35g	8.71g	17.42g
(7) Sodium nitrate	NaNO3	42g	85g	170g
(8) Calcium chloride	CaCl2 2H2O	7.35g	14.7g	29.41g
(9) Magnesium chloride	MgCl2 6H2O	10.16g	20.33g	40.67g
(10) Magnesium sulfate	MgSO4 7H2O	12.33g	24.65g	49.3g

**Because of the small amount needed, cupric sulfate should be made up at a 10x concentration and then diluted. Make a 0.0186g/L cupric sulfate solution and add 10mL to #4.
Store solutions at 4-6°C in polyethylene bottles.
A fresh batch of EDTA should be made every 6 months. Other solutions should be made fresh each year. When new solutions are made, containers should be scrubbed, rinsed, acid washed and rinsed well with DI water.

Algal media is made in either 5L or 2L bottles using 10 stock solutions and MilliQ water. The stock solutions are stored in the refrigerator and made as needed. Stock solutions should be remade every 1 year, with the exception of EDTA which should be remade every 6 months.
1)Fill bottles to 'shoulder' with fresh MilliQ water.
2)Using a clean Eppendorf repeater pipette for the stock solutions. **Never put the Eppendorf pipette into the bottle of stock solution. Instead, pour the appropriate amount of stock solution into a clean beaker.** Combine 1 mL/L of stock solution #1-5 IN NUMERICAL ORDER into a small beaker. Each bottle of medium you are making gets its own beaker. For each of the 5L bottles, you will use 5mL of each stock solution and for each 2L bottle you will use 2mL of each stock solution. Swirl well and let sit for 1 minute. Add each beaker to its own media bottle. There is no need to rinse the pipette between stock solutions.
3)Use the Eppendorf pipette to add 1 mL/L of stock solutions #6-10 directly to the media bottle (ie, 5mL of each stock solution for each 5L bottle and 2mL of stock solution for each 2L bottle).
4)Cover tightly with aluminum foil and place in an autoclavable container with about an inch of water.
5)Autoclave on the liquid setting for 30 minutes at 121°C.After autoclaving, remove and let cool. Check to be sure that precipitation of nutrients has not occurred.

6)When cool, attach to chemostat system.
7)Vitamins should not be autoclaved. Add separately each time new media is added. Use 1mL/L.