MLA Medium Preparation V.2

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This recipe is used to grow algae in the genus Anabaena, Dolichospermum, and Aphanizomenon in the Duffy lab. It can be used to grow other freshwater cyanobacteria cultures.

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Written by: K. Hunsberger, February 2016
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Based on methods from Australian National Algae Culture Collection

MLA Medium Preparation

Summary

This recipe is used to grow algae in the genus *Anabaena, Dolichospermum, and Aphanizomenon* in the Duffy lab. It can be used to grow other freshwater cyanobacteria cultures.

Materials

A	B	C
Apparatus & Equipment	**Labware**	**Reagents**
Autoclave	1L polyethylene bottles	Sodium nitrate, $NaNO_3$
Aluminum foil	Graduated cylinder	Calcium chloride, $CaCl_2 \cdot 2H_2O$
Autoclave tape	500 mL beakers	Magnesium Sulfate, $MgSO_4 \cdot 7H_2O$
Analytical balance	stirring magnet	Potassium phosphate dibasic, K_2HPO_4
Weight boats or paper	5L glass aspirator bottle	Boric acid, H_3BO_3
	Rubber cap for 5L	Selenious acid, H_2SeO_3
	Rubber hose	Copper (II) sulfate, $CuSO_4 \cdot 5H_2O$
	spatula	Iron (III) chloride, $FeCl_3$
		Zinc sulfate, $ZnSO_4 \cdot 7H_2O$
		Cobalt (II) chloride, $CoCl_2 \cdot 6H_2O$
		Sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$
		EDTA, Na_2EDTA
		Sodium bicarbonate, $NaHCO_3$
		Manganese (II) chloride, $MnCl_2 \cdot 4H_2O$

1. Stock Solutions Set #1: Using the analytical balance, weight the prescribed amounts of each of chemicals in the table below. Dissolve the prescribed amount of each chemical into 1 L (final volume) of MilliQ water. There will be 5 separate solutions. Store in polyethylene bottles.

2. Vitamin Solution: Use the solution in the fridge labeled *VIM*. (This VIM solution is from the Standard COMBO Medium Protocol and contains Vitamin B_{12}, Biotin, and Thiamine-HCl. See protocol for specific concentrations.)

3. Micronutrient Primary Stocks: First prepare these 4 primary stocks by adding the prescribed amount to MilliQ water for a final volume of 1L. There will be 4 separate solutions.

A	B	C
Compound	***Symbol***	***g/L MilliQ***
Copper (II) sulfate	**CuSO4 · 5H2O**	1.0
Zinc sulfate	**ZnSO4 · 7H2O**	2.2
Cobalt (II) chloride	**CoCl2 · 6H2O**	1.0
Sodium molybdate	**Na2MoO4 · 2H2O**	0.6

4. Micronutrient Stock Solutions: Dissolve the prescribed amount of the following constituents in ~800 mL of MilliQ water. Allow each to mix and dissolve completely before adding the next ingredient.

A	B	C
Compound	***Symbol***	***g***
EDTA*	**Na2EDTA**	4.36
Iron (III) chloride	**FeCl3 · 6H2O**	1.58
Sodium bicarbonate	**NaHCO3**	0.60
Manganese (II) chloride	**MnCl2 · 4H2O**	0.36

*Add the EDTA first and stir on low heat to fully dissolve.

Then, add 10 of each of the 4 primary stock solutions. Bring the final volume up to 1L. If a precipitate forms, increase the pH up to 7. This solution is the micronutrient stock solution.

5. Stock Solution Set #2: Dissolve the prescribed amount of each chemical into 1 L (final volume) of MilliQ water in 1L flasks. There will be 2 separate solutions.

6.

A	B	C
Compound	***Symbol***	***g/L MilliQ***
Sodium bicarbonate	**NaHCO3**	16.9
Calcium chloride	**CaCl2 · 2H2O**	29.4

Autoclave each of the compounds using the setting number 2 of the BSB autoclaves in floors 4th & 5th(or 121 °C for 15 minutes) and then store in 1L glass bottles.

7. MLA x40 Concentrated Nutrient Solution: Prepare this 1L solution by adding the following volumes of the stock ingredients to 540 mL MilliQ water.

A	B
Solution	***Volume to add***
MgSO4 · 7H2O	40 mL
NaNO3	80 mL
K2HPO4	200 mL
H3BO3	40 mL
H2SeO3	40 mL
VIM Solution	20 mL
Micronutrient stock solution	40 mL

Filter sterilize using a 0.22 mm filter into a sterile 1L glass bottle.

STORE ALL OF THE ABOVE STOCK SOLUTIONS AND PRIMARY STOCK SOLUTIONS IN THE FRIDGE.

Final MLA Medium Preparation: is prepared by adding ~750 mL MilliQ water for each liter. Then, add the correct amount of each solution according to the following table. After adding all of the solutions, the volume should be increased to the specified final amount and adjusted to a pH of 7.5-8 with HCl (if necessary). Prepare final solution in a 1L or 5L aspirator bottle. Place the rubber stopper laterally (so it does not completely plug the top opening of the aspirator bottle) and completely cover the top with aluminum foil. Cover the end of the rubber hose with aluminum foil (or connect it to the second aspirator bottle if setting up a new culture). Autoclave the mixture by selecting program number 2 in the autoclaves found in the 4 or 5th floor of the BSB building (or at 121 °C for 15 min). Allow to cool in autoclave overnight, if possible. This helps to minimize amount of precipitate.

A	B	C
Solution	***Vol. per 1L***	***Vol. per 5L***
Sterile MLA x40 concentrated nutrients	25 mL	125 mL
Sterile NaHCO3	1 mL	5 mL
Sterile CaCl2 · 2H2O	1 mL	5 mL