MLA Medium

Freshwater medium adapted for Cyanobacterial Cultures

Reference: Bolch, C. J. S. and Blackburn S. I. (1996). Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kütz. Journal of Applied Phycology 8: 5-13.

MLA is derived from ASM-1 medium reported in Gorham *etal*, (1964). Isolation and culture of toxic strains of *Anabaena flos-aquae* (Lyngb.) de Bréb. Verh. int. Ver. Limnol 15: 796-804.

	STOCK SOLUTIONS	CONCENTRATION: g L^{-1} DEIONISED WATER (dH_2O)	VOLUME FOR CONCENTRATED NUTRIENT STOCK/MEDIUM
1.	**$MgSO_4.7H_2O$**	49.4 g	10 mL
2.	**$NaNO_3$**	85.0 g	20 mL
3.	**K_2HPO_4**	6.96 g	50 mL
4.	**H_3BO_3**	2.47 g	10 mL
5.	**H_2SeO_3**	1.29 mg	10 mL
6.	**Vitamins**	*see recipe below*	10 mL
7.	**Micronutrients**	*see recipe below*	10 mL
8.	**$NaHCO_3$**	16.9 g	10 mL
9.	**$CaCl_2.2H_2O$**	29.4 g	1 mL

Store all stock solutions in the refrigerator.

Vitamins solution

Add constituents to 100 mL of dH_2O. Store solution in the dark. Remake solution after 3 months.

CONSTITUENT	CONCENTRATION: mg L^{-1} DEIONISED WATER (dH_2O)	QUANTITY FOR WORKING STOCK
Vitamin B_{12}	100 mg	0.05 mL
Biotin	100 mg	0.05 mL
Thiamine HCl	*add reagent directly to stock*	10.0 mg

Micronutrients solution

Add the Na_2EDTA to ~800 mL of dH_2O and stir over low heat to dissolve. Add each of the other constituents separately and fully dissolve between additions. If precipitate forms increase pH up to 7. (If precipitation becomes an issue then replacing the two sulphate stocks with equimolar amounts of the trace metal in the chloride form has proven useful; Ben Long, pers comm)

CONSTITUENT	CONCENTRATION: g L^{-1} DEIONISED WATER (dH_2O)	QUANTITY FOR WORKING STOCK
Na_2EDTA	*add reagent directly to stock*	4.36 g
$FeCl_3.6H_2O$	*add reagent directly to stock*	1.58 g
$NaHCO_3$	*add reagent directly to stock*	0.60 g
$MnCl_2.4H_2O$	*add reagent directly to stock*	0.36 g
$CuSO_4.5H_2O$	1.0 g	10 mL
$ZnSO_4.7H_2O$	2.2 g	10 mL
$CoCl_2.6H_2O$	1.0 g	10 mL
$Na_2MoO_4.2H_2O$	0.6 g	10 mL

MLA Medium Preparation Methods

There are 4 components as follows:

1. Deionised Water

- Autoclave dH_2O to sterilise

2. To prepare MLA x40 concentrated nutrients (250 mL volume)

- Add stock solutions (**1 – 7**) in the quantities stated to 130 mL dH_2O.
- Filter sterilise using a 0.22 µm filter into a sterile 250 mL Schott bottle.

3. $NaHCO_3$

- Prepare stock solution **8** and autoclave at 121°C (15 psi for 20 mins).

4. $CaCl_2.2H_2O$

- Prepare stock solution **9** and autoclave at 121°C (15 psi for 20 mins).

1. To prepare MLA Medium (1 L)

- In a sterile 1000 mL Schott bottle add aseptically:

sterile dH_2O (**1**)	964 mL
sterile MLA x40 concentrated nutrients (**2**)	25 mL
sterile $NaHCO_3$ (**3**)	10 mL
sterile $CaCl_2.2H_2O$ (**4**)	1 mL

- Mix well after each addition.
- This medium is now ready to be decanted aseptically into sterile culture flasks.

2. To prepare MLA Medium - Fully autoclaved (1 L)

For axenic cultures. The media is essentially the same but due to the autoclaving process the $NaHCO_3$ concentration is adjusted.

- In a 1000 mL Schott bottle add:

dH_2O **(1)**	973 mL
sterile MLA x40 concentrated nutrients **(2)**	25 mL
sterile $NaHCO_3$ **(3)**	1 mL
sterile $CaCl_2.2H_2O$ **(4)**	1 mL

- Adjust pH to 7.5 to 8.0 with HCl (often no adjustment is necessary).
- Dispense to flasks and autoclave at 121°C (15 psi, 20 mins).
- Allow to cool in autoclave overnight as this helps to minimise the amount of precipitate.

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