AF-6 Medium

Freshwater medium adapted for Botryococcus

Reference: Provasoli, L. and Pinter, I. J. (1959). Artificial media for fresh-water algae: problems and suggestions. In: Tryon, C. A., Jr. and Hartmann, R. T. (eds.) The Ecology of Algae. Spec. Pub. No. 2, Pymatuning Laboratory of Field Biology, Pittsburgh pp 84-96.

	STOCK SOLUTIONS	CONCENTRATION: g L^{-1} DEIONISED WATER (dH_2O)	QUANTITY FOR MEDIUM
1.	**MES**	*add reagent directly to medium*	400 mg
2.	**$NaNO_3$**	140 g	1.0 mL
3.	**NH_4NO_3**	20 g	1.1 mL
4.	**$MgSO_4.7H_2O$**	80 g	0.375 mL
5.	**KH_2PO_4**	10 g	1.0 mL
6.	**K_2HPO_4**	5 g	1.0 mL
7.	**$CaCl_2.2H_2O$**	29.4 g	0.34 mL
8.	**Fe citrate**	*see recipe below*	0.222 mL
9.	**Biotin**	0.1 g	20 µL
10.	**Thiamine HCl**	0.1 g	0.1 mL
11.	**Vitamin B_6**	0.1 g	10 µL
12.	**Vitamin B_{12}**	0.1 g	10 µL
13.	**P IV Metal Mix**	*see recipe below*	5.0 mL

Store all stock solutions in the refrigerator.

Fe citrate solution

Add both constituents to 1 L of dH_2O and autoclave to dissolve. Store solution in the dark.

CONSTITUENT	QUANTITY
Ferric citrate	9.0 g
Citric acid	9.0 g

P IV Metal Mix

Add EDTA to 1 L of dH_2O and dissolve, then add other constituents to dissolve and autoclave to sterilise. Store solution in the dark.

CONSTITUENT	QUANTITY
$Na_2EDTA.2H_2O$	1.0 g
$FeCl_3.6H_2O$	196 mg
$MnCl_2.4H_2O$	36.0 mg
$ZnSO_4.7H_2O$	22.0 mg
$CoCl_2.6H_2O$	4.0 mg
$Na_2MoO_4.2H_2O$	2.5 mg

To prepare AF-6 Medium (1 L)

- Add MES (**1**) to 990.0 mL dH_2O
- Add each stock solution (**2 – 13**) in the stated volume to the MES solution.
- Adjust the pH to 6.5 – 7 with 20% NaOH.
- Autoclave at 121°C (15 psi for 20 mins).

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