f2 (and fE2) Medium - CSIRO Modification

This medium is a slight modification of the f medium (Guillard and Ryther, 1962) at half strength. The original f medium, when prepared at half strength, is designated as f/2.

References: Jeffrey, S. W. and LeRoi, J.-M. (1997). Simple procedures for growing SCOR reference microalgal cultures. In: S.W. Jeffrey, R.F.C. Mantoura and S.W. Wright (Eds) Phytoplankton pigments in oceanography; Monographs on oceanographic methodology 10, UNESCO, France, pp 181-205.

Guillard, R. R. L. and Ryther, J. H. (1962) Canad. J. Microbiol., 8: 229-239.

	STOCK SOLUTIONS	CONCENTRATION: g L^{-1} DEIONISED WATER (dH_2O)	VOLUME FOR STANDARD MEDIUM	VOLUME FOR CONCENTRATED NUTRIENT STOCK
1.	**$NaNO_3$**	150 g	0.5 mL	5.0 mL
2.	**Trace metals**	*see recipe below*	0.5 mL	5.0 mL
3.	**$Na_2SiO_3.5H_2O$**	22.7 g	0.5 mL	5.0 mL
4.	**Fe citrate**	*see recipe below*	0.5 mL	5.0 mL
5.	**Vitamins**	*see recipe below*	0.5 mL	5.0 mL
6.	**$NaH_2PO_4.2H_2O$**	11.3 g		5.0 mL
7.	**$Na_2EDTA.2H_2O$**	30.0 g		

Store all stock solutions in the refrigerator.

Trace metal solution

Add each of the constituents to ~750 ml dH_2O, mixing thoroughly between additions to dissolve. Make solution up to 1 L.

CONSTITUENT	QUANTITY
$CuSO_4.5H_2O$	19.6 mg
$ZnSO_4.7H_2O$	44.0 mg
$CoCl_2.6H_2O$	22.0 mg
$MnCl_2.4H_2O$	360.0 mg
$Na_2MoO_4.2H_2O$	12.6 mg

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

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	1.0 mL
Biotin	100 mg	1.0 mL
Thiamine HCl	*add reagent directly to stock*	20.0 mg

1. To prepare f2 Medium (1 L)

- Add each stock solution (**1 – 5**) in the Standard quantities to 1 L seawater (0.22µm filtered).
- Dispense to flasks and autoclave at 121°C (15 psi, 20 mins).

Phosphate (see Stock **6** - $NaH_2PO_4.2H_2O$). This must be sterilised separately from seawater to prevent precipitation.

- Dilute original phosphate stock with dH_2O such that 1 mL added to 75 mL of sterile medium will give the required concentration of phosphate (11 mg L^{-1}) in the medium.
- Autoclave dilute phosphate stock at 121°C (15 psi, 20 mins).
- After cooling, dispense aseptically with sterilised automatic dispenser.

For example:

For 125 mL Erlenmeyer flasks, each containing 75 mL medium, prepare dilute phosphate stock as follows:

- **f2 and fE2 media:**
  - Take 3.75 mL of original phosphate stock and make up to 100 mL with dH_2O.
  - Pour into a 250 mL Schott bottle and autoclave to sterilize.
  - Dispense 1 mL per flask aseptically.
- **f and fE media:**
  - Take 7.5 mL of original phosphate stock and make up to 100 mL with dH_2O.
  - Pour into a 250 mL Schott bottle and autoclave to sterilize.
  - Dispense 1 mL per flask aseptically.

Scale dispense volumes to the same proportion for differing medium volumes.

To prepare fE2 Medium (1 L)

Prepare as f2 Medium, but also add 0.5mL of $Na_2EDTA.2H_2O$ stock solution (**7**).

To prepare f Medium (1 L)

Prepare as f2 Medium using 1.0 mL of each stock solution (**1 – 5**) instead of 0.5 mL.

To prepare fE Medium (1 L)

Prepare as f Medium, but also add 1 mL of $Na_2EDTA.2H_2O$ stock solution (**7**).

2. To prepare f2 concentrated nutrients

- Combine each of the stock solutions (**1 – 6**) in the Concentrated quantities and make up to 100 mL with dH_2O.
- Pour into a 250 mL Schott bottle.
- Autoclave at 121°C (15 psi, 20 mins). Alternatively, filter sterilise using a 0.22 µm filter into a sterile 250 mL Schott bottle.

Use 1 mL per 100 mL sterile seawater adding the correct amount of nutrients aseptically

CONTACT US

t 1300 363 400
+61 3 9545 2176
e csiroenquiries@csiro.au
w www.csiro.au

For further information

Australian National Algae Culture Collection
w www.csiro.au/en/Research/Collections/ANACC

Ian Jameson
Director
t +61 3 6232 5117
e ian.jameson@csiro.au

Australian National Algae Supply Service

Cathy Johnston
Manager
t +61 3 6232 5316
e cathy.johnston@csiro.au