AF6 Medium, modified

(Watanabe *et al.* 2000)

This is a good general freshwater medium that supports the growth of a wide diversity of algae. The original medium was designed for growing *Colacium* (Euglenophyceae) (Kato 1982). It has been modified at the NIES Culture Collection of Microalgae and Protozoa (Watanabe *et al.* 2000) by adding MES buffer, removing $CaCO_3$ and substituting a different trace metal solution.

Prepare the stock solutions. To 950 mL of dH_2O, first dissolve the MES and citrate, then add the stock solutions and bring the final volume up to 1 liter. The pH is adjusted to 6.6. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
MES buffer		400 mg	2.05×10^{-3} M
$NaNO_3$	140 g L^{-1} dH_2O	1 mL	1.65×10^{-3} M
NH_4NO_3	22 g L^{-1} dH_2O	1 mL	2.75×10^{-4} M
$MgSO_4$ $7H_2O$	30 g L^{-1} dH_2O	1 mL	1.22×10^{-4} M
K_2HPO_4	5 g L^{-1} dH_2O	1 mL	2.87×10^{-5} M
KH_2PO_4	10 g L^{-1} dH_2O	1 mL	7.35×10^{-5} M
$CaCl_2$ $2H_2O$	10 g L^{-1} dH_2O	1 mL	6.80×10^{-5} M
Fe-citrate	2 g L^{-1} dH_2O	1 mL	8.17×10^{-6} M
Citric acid	2 g L^{-1} dH_2O	1 mL	1.04×10^{-5} M
trace metals solution	(see below)	1 mL	---
Vitamin solution	(see below)	1 mL	---

Trace Metals Solution

This trace metals solution is a modified version of PIV trace metals solution (Provasoli and Pintner 1960). Prepare the primary stock solutions. Into 950 mL of dH_2O, pH EDTA to 8.0-8.4 to assure it fully dissolves, add Fe and fully, and finally add 1 mL of each primary stock.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
$Na_2EDTA\ 2H_2O$	---	5.000 g	1.34×10^{-5} M
$FeCl_3\ 6H_2O$	---	0.98 g	3.63×10^{-6}M
$MnCl_2\ 4H_2O$	180 g L^{-1} dH_2O	1 mL	9.10×10^{-7} M
$ZnSO_4\ 7H_2O$	110 g L^{-1} dH_2O	1 mL	3.83×10^{-7} M
$CoCl_2\ 6H_2O$	20.0 g L^{-1} dH_2O	1 mL	8.41×10^{-8}M
$Na_2MoO_4\ 2H_2O$	12.5 g L^{-1} dH_2O	1 mL	5.17×10^{-8} M

AF6 Vitamin Solution

First, prepare the three primary stocks. Into 950 mL of dH_2O, add and dissolve the thiamine, and then add 1 mL from each of the two primary stocks. Filter sterilize. Refrigerate or freeze.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine (vit. B_1)	---	10 mg	2.96×10^{-8} M
biotin (vit. H)	2.0 g L^{-1} dH_2O	1 mL	8.19×10^{-9} M
cyanocobalomin (vit. B_{12})	1.0 g L^{-1} dH_2O	1 mL	7.38×10^{-10} M
pyridoxine (vit. B_6)	1.0 g L^{-1} dH_2O	1 mL	5.91×10^{-9} M

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Provasoli, L. and Pintner, I.J. 1960. Artificial media for fresh-water algae: problems and suggestions. pp. 84-96. *In* Tyron, C.A. Jr. and Hartman, R.T. (eds.) *The Ecology of Algae*. Special Publ. 2, Pymatuning Laboratory of Field Biology, Univ. Pittsburgh, PA.

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As of 2017 the NCMA has used standard f/2 vitamins without deleterious effects.

f/2 Vitamin Solution

(Guillard and Ryther 1962, Guillard 1975)

First, prepare primary stock solutions. To prepare final vitamin solution, begin with 950 mL of dH_2O, dissolve the thiamine, add the amounts of the primary stocks as indicated in the quantity column below, and bring final volume to 1 liter with dH_2O.At the NCMA we autoclave to sterilize. Store in refrigerator or freezer.

Component	PrimaryStock Solution	Quantity	Molar Concentration in Final Medium
thiamine · HCl (vit. B_1)	---	200 mg	2.96×10^{-7} M
biotin (vit. H)	0.1 g/ L dH_2O	10 mL	2.05×10^{-9} M
cyanocobalamin (vit. B_{12})	1.0 g/ L dH_2O	1 mL	3.69×10^{-10} M

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