YBC-II Medium

(Chen et al. 1996)

This artificial seawater medium was developed to culture the nitrogen fixing *Trichodesmium* (no nitrogen source in medium) under critical culture conditions for physiological studies. It was based upon Ohki's medium (Ohki et al. 1992), but it is significantly different. Another more complex medium (YBC-III) was presented in Chen et al. (1996), and it more closely resembles oligotrophic seawater.

To prepare, make the necessary stock solutions using high quality dH_2O. Beginning with 900 mL of dH_2O, add the following components in the quantities provided. Bring the final volume to 1 liter with dH_2O. Adjust to pH 8.15 to 8.2 with NaOH and filter sterilize; do not autoclave.

Component	Stock Solution	Quantity	Final Molar Concentration in Medium
anhydrous salts			
NaCl	---	24.5500 g	4.20×10^{-1} M
KCl	---	0.7500 g	1.00×10^{-2} M
$NaHCO_3$	---	0.2100 g	2.5×10^{-3} M
H_3BO_3	---	0.0360 g	5.80×10^{-4} M
KBr	---	0.1157 g	9.72×10^{-4} M
NaF	2.94 g L^{-1} dH_2O	1 mL	7.0×10^{-5} M
hydrous salts			
$MgCl_2 \cdot 6H_2O$	---	4.067 g	2.00×10^{-2} M
$CaCl_2 \cdot 2H_2O$	---	1.47 g	1.00×10^{-2} M
$MgSO_4 \cdot 7H_2O$	---	6.16 g	2.50×10^{-2} M
$SrCl_2 \cdot 6H_2O$	17.33 g L^{-1} dH_2O	1 mL	6.50×10^{-8} M
macronutrient			
$NaH_2PO_4 \cdot H_2O$	6.9 g L^{-1} dH_2O	1 mL	5.00×10^{-5} M
trace metals solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	1 ml	---

Trace Metal Solution

Prepare the necessary primary stock solutions. To Prepare the final trace metal mixture, begin with 950 mL of dH_2O, add the EDTA and 1 mL of each primary stock solution. Filter sterilize.

Component	Primary Stock Solution	Quantity	Final Molar Concentration in Medium
$Na_2EDTA \cdot 2H_2O$	---	0.745 g	2.00×10^{-6} M
$FeCl_3 \cdot 6H_2O$	0.11 g L^{-1} dH_2O	1 mL	4.07×10^{-7} M
$MnCl_2 \cdot 4H_2O$	3.96 g L^{-1} dH_2O	1 mL	2.00×10^{-8} M
$ZnSO_4 \cdot 7H_2O$	1.15 g L^{-1} dH_2O	1 mL	4.00×10^{-9} M
$CoCl_2 \cdot 6H_2O$	5.95 g L^{-1} dH_2O	1 mL	2.50×10^{-9} M
$Na_2MoO_2 \cdot 2H_2O$	2.66 g L^{-1} dH_2O	1 mL	1.10×10^{-8} M
$CuSO_4 \cdot 5H_2O$	2.23 g L^{-1} dH_2O	1 mL	1.00×10^{-9} M

Vitamin Solution

Prepare the necessary primary stock solutions. To prepare the final vitamin solution, begin with 950 mL of dH_2O, add 100 mg of thiamine and 1 mL of each primary stock solution. Bring the final volume to 1 liter with dH_2O. Filter sterilize and store frozen.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine • HCl	---	100 mg	2.96×10^{-7}
biotin	0.5 g L^{-1} dH_2O	1 mL	2.05 x 1
cyanocobalamin	0.5 g L^{-1} dH_2O	1 mL	3.69×10^{-10}

Chen, Y.-B., Zehr, J.P. & Mellon, M. 1996. Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101 in defined media: evidence for a circadian rhythm. *J. Phycol*. **32**: 916-923.

Ohki, K., Zehr, J.P. & Fujita, Y. 1992. *Trichodesmium*: establishment of culture and characteristics of N-fixation. pp. 307-318. *In* Carpenter, E.J., Capone, D.G. & Rueter, J.G. [eds.] Marine Pelagic Cyanobacteria: *Trichodesmium* and other diazotrophs. Kluwer Acad. Publ., Dordrecht.

Starr and Zeikus 1993

This medium, modified from Provasoli and Pintner (1960), is useful for growing many freshwater algae (Starr and Zeikus 1993).

To prepare, begin with 900 mL dH_2O, dissolve the MES and calcium nitrate, add the other stock solution quantities, and after thorough mixing, add the vitamins. Adjust pH to 6.7 with NaOH. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
MES	---	1.95 g	1.00×10^{-2} M
$Ca(NO_3)_2$ $4H_2O$	---	117.8 mg	5.00×10^{-4} M
Na_2 b-glycerophosphate $5H_2O$	60.0 g L^{-1} dH_2O	1 mL	1.96×10^{-4} M
$MgSO_4$ $7H_2O$	40.0 g L^{-1} dH_2O	1 mL	1.62×10^{-4} M
KCl	50.0 g L^{-1} dH_2O	1 mL	6.71×10^{-4} M
NH_4Cl	26.7 g L^{-1} dH_2O	1 mL	5.00×10^{-4} M
trace metals solution	(see recipe below)	6 mL	---
biotin (vit. H)	2.5 mg L^{-1} dH_2O	1 mL	1.02×10^{-8} M
cyanocobalomin (vit. B_{12})	1.5 mg L^{-1} dH_2O	1 mL	1.11×10^{-9} M

Trace Metals Solution

Starr and Zeikus (1993) refer to this as PIV trace metals solution, but it differs slightly from the original PIV trace metals (see Volvox Medium) (Provasoli and Pintner 1960). Prepare the primary stock solution. Into 950 mL of dH_2O, dissolve the EDTA and then individually add and dissolve the metals. Bring to 1 liter with dH_2O.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
Na_2EDTA	---	750 mg	1.54×10^{-5} M
$FeCl_3\ 6H_2O$	---	97 mg	2.15×10^{-6} M
$MnCl_2\ 4H_2O$	---	41 mg	1.24×10^{-6} M
$ZnCl_2$	5.0 g L^{-1} dH_2O	1 mL	2.20×10^{-7} M
$Na2MoO_4\ 2H_2O$	4.0 g L^{-1} dH_2O	1 mL	9.92×10^{-8} M
$CoCl_2\ 6H_2O$	2.0 g L^{-1} dH_2O	1 mL	5.04×10^{-8} M

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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