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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 (YBCIII) was presented in Chen et al. (1996), and it more closely resembles oligotrophic seawater. To prepare, make the necessary stock solutions using high quality dH2O. Beginning with 900 mL of dH2O, add the following components in the quantities provided. Bring the final volume to 1 liter with dH2O. Adjust to pH 8.15 to 8.2 with NaOH and filter sterilize; **do not** autoclave.

Weigh out and add the following to 900mL of high quality dH_2O:

Component	Grams per liter	Concentration (in moles) in YBCII
NaCl	24.55	$4.20X10^{-1}$
KCl	0.75	$1.00X10^{-2}$
$NaHCO_3$	0.21	$2.50X10^{-3}$
H_3BO_3	0.036	$5.80X10^{-4}$
KBr	0.1157	$9.72X10^{-4}$
$MgCl_2 \cdot 6H_2O$	4.07	$2.00X10^{-2}$
$CaCl_2 \cdot 2H2O$	1.47	$1.00X10^{-2}$
$MgSO_4 \cdot 7H_2O$	6.18	$2.50X10^{-2}$

Make the following primary (1°) stock solutions and add the recommended amounts to the above 900mL of high quality dH_2O:

Component	Grams per one liter of 1° stock solution	Concentration in moles in 1° stock solution	Amount of 1°stock solution to add to 1L of medium	Final concentration in moles in 1L of YBCII medium
NaF	2.94/liter	$7.00X10^{-2}$	1mL	$7.00X10^{-5}$
$SrCl_2 \cdot 6H_2O$	17.4g/liter	$6.50X10^{-2}$	1mL	$6.50X10^{-5}$
KH_2PO_4	6.8g/liter	$5.00X10^{-2}$	1mL	$5.00X10^{-5}$
Na_2EDTA	0.74g/liter	$2.00X10^{-3}$	1mL	$2.00X10^{-6}$
$FeCl_3 \cdot 6H_2O$	0.11g/liter	$4.07X10^{-4}$	1mL	$4.07X10^{-7}$
$MnCl_2 \cdot 4H_2O$	0.04g/liter	$2.00X10^{-4}$	100µL	$2.00X10^{-8}$
$ZnSO_4 \cdot 7H_2O$	0.012g/liter	$4.00X10^{-5}$	100µL	$4.00X10^{-9}$
$Na_2MoO_4 \cdot 2H_2O$	0.027g/liter	$1.1X10^{-4}$	100µL	$1.10X10^{-8}$
$CoCl_2 \cdot 6H_2O$	0.06g/liter	$2.5X10^{-4}$	10µL	$2.50X10^{-9}$

$CuSO_4 \cdot 5H_2O$	0.025g/liter	$1.00X10^{-4}$	10µL	$1.00X10^{-9}$
f/2 vitamins			1.2mL	See recipe below

f/2 Vitamin Solution

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	Primary Stock Solution	Quantity	Molar Concentration in Final Medium when used at 1mL per liter
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

Chen, Yi-Bu, Jonathan P. Zehr, and Mark Mellon. "Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium Trichodesmium sp. IMS 101 in defined media: evidence for a circadian rhythm." *Journal of Phycology* 32.6 (1996): 916-923.

Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. pp 26-60. *In* Smith W.L. and Chanley M.H (Eds.) *Culture of Marine Invertebrate Animals*. Plenum Press, New York, USA.

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