Media recipes for Synechococcus isolates

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Abstract

The following are media recipes necessary for isolating and maintaining *Synechococcus* isolates.

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Guidelines

SN MEDIUM (used for maintenance of culture stocks)

75% Seawater	(750 mL)
25% Milli-Q Water	(250 mL)

SYN Stocks

Reagent	Amount	Stock Concentration
EDTA*	5 mL/L	1 g/L
K_2PO_4[1]	2.5 mL/L	6.1 g/L (anhydrous)
$NaNO_3$	2.5 mL/L	30%
Na_2CO_3	0.5 mL/L	20 g/L
Cyano TMM*	1 mL/L	see recipe

[1] Na_2HPO_4 OK also
* Matt's solution uses 0.1 mL/L & contains EDTA

2/23/09: for WH6501 make up CEDO-PRO99 supplement w/ NH_4Cl, $NaNO_3$, Na_2HPO_4, and CTMM (100 µL/L)

Notes: May need to add VA Vitamin mix as necessary if cultures are pure (and lacking the heterotrophic bacteria contaminants that might make and provide the vitamins necessary) - 1 drop/50mL flask

May also choose to do SN/10 by dividing the recipe by 10 and leaving out the nitrogen source to obtain a medium useful for isolating clonal cultures via serial dilution from natural seawater samples.

SNX: nitrate PLUS ammonium as nitrogen source
add 1 mL/L of $NaNO_3$ stock, plus 0.5 mL/L NH_4Cl stocks

SNY: just ammonium as nitrogen source
add 0.5 mL/L NH_4Cl stock

SU: urea as the nitrogen source
add 1 mL/L of 1M urea stock (60g urea/L=1M urea stock)

For plates add sodium sulfate (NOTE: will get ~40-50 plates per liter of medium)
-Add 0.35g Baculovirus agar per 100 mL SN/10 to get about 10 plates

Can add more Carbonate (~3x more) to obtain cultures of Syn that are better for DNA studies

SNAX MEDIUM

75% Seawater
25% Milli-Q Water

SN stock reagents as follows:

Reagent	Amount	Stock Concentration
EDTA	0.5 mL/L	1 g/L
K_2PO_4	0.25 mL/L	6.1 g/L (anhydrous)
$NaNO_3$	0.25 mL/L	30%
Na_2CO_3	0.25 mL/L	4 g/L
Cyano TMM	0.1 mL/L	see recipe
Ammonium Chloride 1M Stock	1 mL/L	1M

For plates add sodium sulfate

CYANO TRACE METALS MIX (Cyano TMM) for 'SN'. 'SNX', 'SNY', 'ASW', plates and slants

$ZnSO_4 \cdot 7H_2O$	0.222 g/L	[free Zn++]=0.192 mg/L
$MnCl_2 \cdot 4H_2O$	1.4 g/L	[free Mn++]=0.39 mg/L
$Co(NO_3) \cdot 6H_2O$	0.025 g/L	[free Co++]=0.005 mg/L
$Na_2MoO_4 \cdot 2H_2O$	0.39 g/L	[free Mo]=0.15 mg/L
Citric acid$\cdot H_2O$	6.25 g/L	
Ferric Ammonium Citrate	6.0 g/L	[free Fe+++]=1.05 mg/L

VA VITAMIN MIX

Vitamins	Primary Stock	To make solution
B-12	1 mg/mL	0.1 mL/100 total
Thiamine (fridge)	---	weigh 200 mg/100 total
Biotin (fridge)	0.1 mg/mL	1 mL/100 total
Paba	2 mg/mL	0.5 mL/100 total
Folic Acid	1 mg/mL	0.1 mL/100 total
Niacin	1 mg/mL	10 mL/100 total
Inositol	---	weigh 100 mg/100 total
Ca pantothenate	2 mg/mL	10 mL/100 total
Pyridoxine	1 mg/mL	10 mL/100 total

NOTES: Keep stocks and vitamin solutions frozen. Filter sterilize through a 0.2 µm Nucleopore filter.

Do purity test on 1 tube to ensure that it is sterile before adding to stocks.

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