Making AA+ medium for Synechococcus sp. PCC 7002

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Abstract

This protocol discribes how to make growth medium for Synechococcus sp. PCC 7002, including the stock solutions needed.

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Protocol

Make stock solutions

Step 1.

Make 1000x $FeCl_2x6H_2O$

- Add 0.405g $FeCl_2x6H_2O$ to 100mL MilliQ
- Filtersterilize. **Do not autoclave!**

Step 2.

Make 100x Tris 8.2

- 9.99g Tris in 100mL MilliQ
- Adjust pH to 8.2 with HCl
- Autoclave

Step 3.

Make 1000 x Trace mineral solution

- Use the following compounds:

Compound	Amount (g/L)
H_3BO_3	2.86
$MnCl_2$ x 4 H_2O	1.81
$ZnSO_4$ x 7 H_2O	0.222
Na_2MoO_4 x 2 H_2O	0.390
$CuSO_4$ x 5 H_2O	0.079
$Co(NO_3)_2$ x 6 H_2O	0.0494

- Autoclave or filtersterilize

Step 4.

Make 100x AA+ solution

- Use the following compounds, autoclave

Compound	500mL	250mL	100mL
KH_2PO_4	1.25g	0.625g	0.25g
Na_2-EDTA x 2 H_2O	1.5g	0.750g	0.3g

Compound	500mL	250mL	100mL
KCl	30g	15g	6g
$NaNO_3$	50g	25g	10g
CaCl2	6.65g	3.325g	1.33g

Make the AA+ medium

Step 5.

- Add compounds as described in table below to desired amount of miliQ.
- Add glycerol only when non-phototrophic growth is needed

Compound	1L	500mL	250mL
NaCl	18g	9g	4.5g
$MgSO_4x7H_2O$	5g	2.5g	1.25g
Glycerol	1.1mL	550µL	275uL

- If you want to make plates, add 15g/L agar agar
- Autoclave

Step 6.

- Add compounds (table below) in the flow cabinet to the autoclaved and *hand-warm* solution
- Only add the B_{12} if you are immediately using the medium. If some medium with B_{12} is left after you used it, store it in a dark, cold place for a short time

Compound	1L	500mL	250mL
100x Tris pH 8.2	10mL	5mL	2.5mL
1000x $FeCl_2x6H2O$	1mL	500µL	250µL
1000x Trace mineral solution	1mL	500µL	250µL
100x AA+ solution	10mL	5mL	2.5mL
vit B12 (1mg/mL)	4µL	2µL	1µL