

CT Media Version 2

Dr. Steven Wilhelm

Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

Modified from: Watanabe, Makoto M. "Fresh-and salt-water forms of *Spirulina platensis* in axenic cultures." *Bull. Jpn. Soc. Phycol.* 25 (1977): 371-377.

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Protocol

Stock Solutions Preparation

Step 1.

Add 20 g/100 mL TAPS in an acid-washed bottle

Stock Solutions Preparation

Step 2.

Add 10 g/100 mL KNO₃ in an acid-washed bottle



REAGENTS

✓ Potassium nitrate by Contributed by users

Stock Solutions Preparation

Step 3.

Add 16 g/100 mL Ca(NO₃)₂*4H₂O



REAGENTS

✓ calcium nitrate by Contributed by users

Stock Solutions Preparation

Step 4.

Add 4 g/100 mL MgSO₄*7H₂O



REAGENTS



Magnesium sulfate heptahydrate by Contributed by users

Stock Solutions Preparation

Step 5.

Add 5 g/100 mL $\text{Na}_2\text{beta-glycerophosphate}$

Trace Metals Stock

Step 6.

Add 1 L Milli-Q H_2O to an acid-washed bottle

Trace Metals Stock

Step 7.

Add 1 g Na_2EDTA



REAGENTS



EDTA Disodium Salt [PubChem CID: 8759](#) by Contributed by users

Trace Metals Stock

Step 8.

Add 194 mg FeCl_3



REAGENTS

Iron(III) chloride hexahydrate [44944](#) by [Sigma Aldrich](#)

Trace Metals Stock

Step 9.

Add 36 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$



REAGENTS

Manganese chloride 7773-01-5 by [Fisher Scientific](#)

Trace Metals Stock

Step 10.

Add 10.44 mg ZnCl_2



REAGENTS



Zinc dichloride by Contributed by users

Trace Metals Stock

Step 11.

Add 12.62 mg $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$



REAGENTS



Sodium molybdate dihydrate by Contributed by users

Trace Metals Stock

Step 12.

Add 4.04 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$



REAGENTS

Cobalt (II) chloride hexahydrate 7791-13-1 by [Fisher Scientific](#)

Trace Metals Stock

Step 13.

Filter sterilize. It may be helpful to dispense into 50 mL Falcon tubes. Store at 4°C.

Vitamins Stock

Step 14.

Add 1 L Milli-Q H_2O to an acid-washed bottle

Vitamins Stock

Step 15.

Add 10 mg Thiamine HCl (Vitamin B1)



REAGENTS



Thiamine HCl [View](#) by [P212121](#)

Vitamins Stock

Step 16.

Add 0.1 mg Biotin (Vitamin H)



REAGENTS



Biotin [View](#) by [P212121](#)

Vitamins Stock

Step 17.

Add 0.1 mg Cyanocobalamin (Vitamin B12)



REAGENTS

Vitamin B12 68-19-9 by [Fisher Scientific](#)

Vitamins Stock

Step 18.

Filter sterilize. Aliquot into 1.5 mL microcentrifuge tubes and store and -20°C.

Media Additions

Step 19.

Add 990 mL Milli-Q H₂O to an acid-washed bottle

Media Additions

Step 20.

Add 2 mL TAPS stock solution

Media Additions

Step 21.

Add 1 mL KNO₃ stock solution

Media Additions

Step 22.

Add 1 mL Ca(NO₃)₂*4H₂O stock solution

Media Additions

Step 23.

Add 1 mL MgSO₄*7H₂O stock solution

Media Additions

Step 24.

Add 1 mL N₂*beta-glycerophosphate stock solution

Media Additions

Step 25.

Add 3 mL trace metal stock solution

Media Additions

Step 26.

Adjust pH to 8.2

Media Additions

Step 27.

Autoclave at 121°C for 20 min

Media Additions

Step 28.

Add 1 mL vitamin stock solution after media cools