

Fraquil Media

Dr. Steven Wilhelm

Abstract

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

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Protocol

Individual Salt Stock Solutions

Step 1.

Add 18.4 g $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ to 500 mL Milli-Q H_2O in a clean polyethylene bottle



REAGENTS

✓ Calcium Chloride by Contributed by users

Step 2.

Add 18.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ to 500 mL Milli-Q H_2O in a clean polyethylene bottle



REAGENTS

✓ Magnesium sulfate heptahydrate by Contributed by users

Step 3.

Add 6.3 g NaHCO_3 to 500 mL Milli-Q H_2O in a clean polyethylene bottle



REAGENTS

✓ Sodium bicarbonate [View](#) by [P212121](#)

Step 4.

Add 0.87 g K_2HPO_4 to 500 mL Milli-Q H_2O in a clean polyethylene bottle



REAGENTS

✓ Potassium phosphate (dibasic) [View](#) by [P212121](#)

Step 5.

Add 4.25 g NaNO_3 to 500 mL Milli-Q H_2O in a clean polyethylene bottle



REAGENTS



Sodium nitrate [View](#) by [P212121](#)

Trace Metals Mix (1 uM Fe final stock)

Step 6.

Add 443 mL Milli-Q H₂O to a clean polyethylene container

Step 7.

Add 500 µL CuSO₄*5H₂O



REAGENTS



Copper Sulfate [View](#) by [P212121](#)

Step 8.

Add 500 µL (NH₄)₆Mo₇O₂₄*4H₂O



REAGENTS

Ammonium molybdate (VI) tetrahydrate 12054-85-2 by [Fisher Scientific](#)

Step 9.

Add 500 µL CoCl₂*6H₂O



REAGENTS

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

Step 10.

Add 500 µL MnCl₂*4H₂O



REAGENTS

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

Step 11.

Add 500 µL ZnSO₄*7H₂O



REAGENTS



Zinc sulfate by Contributed by users

Step 12.

Add 5 mL Na₂EDTA (0.5 M)



REAGENTS



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

Step 13.

Add 50 mL $\text{FeCl}_3 \cdot 7\text{H}_2\text{O}$

REAGENTS

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

F/2 Vitamin Solution

Step 14.

Add 1 L Milli-Q H_2O to a clean bottle

Step 15.

Add 1 mL vitamin B_{12} (1.0 g/L d H_2O)

Step 16.

Add 10 mL Biotin (0.1 g/L d H_2O)

REAGENTS

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

Step 17.

Add 200 mg Thiamine HCl

REAGENTS

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

Media additions

Step 18.

Add 993 mL Milli-Q H_2O to a clean polycarbonate bottle

Step 19.

Add 1 mL CaCl_2

Step 20.

Add 1 mL MgSO_4

Step 21.

Add 1 mL NaHCO_3

Step 22.

Add 1 mL K_2HPO_4

Step 23.

Add 1 mL NaNO_3

Step 24.

Add 1 mL Trace metal mix

Step 25.

Add 1 mL f/2 vitamin mix

Step 26.

Filter sterilize and dispense into acid-washed/microwave tyndalized polycarbonate tubes.