Saltwater BG-11 recipe

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Abstract

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Protocol

Step 1.

Stock solutions:

Macronutrient Stock Solutions:

Make each stock solution seperately. Prepare each in 32 ppt seawater.

150 g/L $NaNO_3$

4 g/L $MgSO_4 * 7H_2O$

3.6 g/L $CaCL_2 * 2H_2O$

0.6 g/L Citric Acid $* 2H_2O$

0.6 g/L Ferric Ammonium Citrate

0.1 g/L $Na_2EDTA * 2H_2O$

2 g/L $NaCO_3$

Make each stock solution seperately. Prepare each in ddH20**.**

4 g/L K_2HPO_4

30 g/L $NaSiO_3$

Trace Metal Stock Solution:

Combine ingredients in ddH2O.

2.86 g/L H_3BO_3

1.81 g/L $MnCL_2$ * $4H_2O$

0.22 g/L $ZnSO_4$ * $7H_2O$

0.39 g/L Na_2MoO_4 * $2H_2O$

0.79 g/L $CuSO_4$ * $5H_2O$

0.50 g/L $Co(NO_3)_2$ * $6H_2O$

Vitamin Stock Soluiton:

Combine ingredients in ddH2O.

0.135 g/L Vitamin B_{12} (cyanocobalamin)

0.025 g/L Biotin

0.335 g/L Thiamine

12 g/L HEPES buffer pH 7.8

Step 2.

Prepare media:

To 750mL of 32ppt seawater add:

- 10 ml of each Macronutrient Stock Solution for each litre of BG-11 being made. Be sure to add K_2HPO_4 first, then $NaSiO_3$, then add the others. K2HPO4 and NaSiO3 must be added first to prevent precipitation.

- 1ml of the Trace Metal Stock Solution for each litre of BG-11 being made.

- 1ml of the Vitamin Stock Solution for each litre of BG-11 being made.

Adjust pH of the final media to 8.2.

Step 3.

0.2 um filter sterilize into clean, autoclaved containers in a biosafety hood. Do not autoclave the media as the silica will form precipitate and be cloudy!

Step 4.

To Prepare Conjugation Plates (1/2 BG-11, 5% LB, 1% agar):

1. Dilute Saltwater BG-11 1:1 with ddH_2O.
2. Add 50mL of 1x LB for each litre of Conjugation Plate media being made.
3. Add 5g of Bacto Agar to a 1L Earlenmeyer flask for each 500mL of Conjugation Plate media being made.
4. Distribute 500 mL of Conjugation Plate media to each 1L Earlenmeyer flask.
5. Cover with flask opening with aluminum foil and autoclave at 121C for 30 minutes.
6. Allow media to cool while stirring on magnetic stir plate. Make sure the stirring is not too vigorous as to cause bubbles. It should cool enough to be able to touch, but not have the agar solidified.
7. Pour plates and allow to cool overnight.

Step 5.

To Prepare Selection Plates (1/2 BG-11, 1% agar, + antibiotics):

1. Dilute Saltwater BG-11 1:1 with ddH_2O.
2. Add 5g of Bacto Agarto to a 1L Earlenmeyer flask for each 500mL of Selection Plate media being made.
3. Distribute 500 mL of Selection Plate media to each 1L Earlenmeyer flask.
4. Cover flask opening with aluminum foil and autoclave at 121C for 30 minutes.
5. Allow media to cool while stirring on magnetic stir plate. Make sure the stirring is not too vigorous as to cause bubbles. It should cool enough to be able to touch, but not have the agar solidified.
6. Add 500ul of the required antibiotics to the Selection Plate media made. We make 500uL aliquots of 1000x antibiotic stocks. It is very important that the media is cool before adding the antibiotics or the heat will destroy them.
7. Pour plates and allow to cool overnight.