Saltwater BG-11 recipe Version 3

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Abstract

Preparation of BG-11 growth medium.

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Protocol

Step 1.

Stock solutions

Macronutrient Stock Solutions:

Prepare each of the following stock solutions seperately 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 $NaCO_3$

Prepare each of the following stock solutions seperately in ddH_20**.**

4 g/L K_2HPO_4

30 g/L $NaSiO_3$

Note: Be sure to prepare K_2HPO_4 and $NaSiO_3$ stock solutions in dd H_2O to prevent precipitation.

Trace Metal Stock Solution:

Combine these ingredients in dd H_2O.

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 * $2H_2O$

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

0.0494 g/L $Co(NO_3)_2$ * $6H_2O$

Vitamin Stock Soluiton:

Combine ingredients in dd H_2O.

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:

Note: Add ingredients in the following order to avoid precipitation. Add the K_2HPO_4 stock solution first. Then add the $NaSiO_3$ stock solution. Add the other macronutrient stock solutions last.

For each liter of BG-11:

- 750mL of 32 ppt seawater
- 10 ml of K_2HPO_4 Macronutrient Stock Solution

- 1 mL of $NaSiO_3$ Macronutrient Stock Solution
- 10 mL of **each** Macronutrient Stock Solution
- 1 ml of the Trace Metal Stock Solution
- 1 ml of the Vitamin Stock Solution

Bring final volume to 1 L.

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 a cloudy white precipitate.

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 5 g of Bacto Agar to a 1 L 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 a 1 L Erlenmeyer flask for each 500 mL of Selection Plate media being made.
3. Distribute 500 mL of Selection Plate media to each 1 L Erlenmeyer flask.
4. Cover flask opening with aluminum foil and autoclave at 121 C 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 500 ul of the required antibiotics to the Selection Plate media made. We make 500uL aliquots of 1000x antibiotic stocks. Heat will destroy the antibiotics, so it is very important that the media is cool before adding the antibiotics.

7. Pour plates and allow to cool overnight.