Marine Purity Broth

Bonnie Poulos

Abstract

Purpose: To test axenic cultures for purity.
Protocol described in S. Bertillson, O. Berglund, D.M. Karl, S.W. Chisholm (2003). Elemental composition of marine Prochlorococcus and Synechococcus: Implications for the ecological stoichiometry of the sea. Limnol Oceanogr 48(5):1721-1731.

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Guidelines

Formulation:

17 g BD Difco AC Broth
20 g NaCl
Qs to 1 L with distilled H_2O

After broth has cooled:

2 M $MgSO_4$ (2 M $MgSO_4 \cdot 7H_2O$ = 49.3 g/100 ml H_2O, dissolve and 0.2 µm filter)
1 M $CaCl_2$ (1 M $CaCl_2 \cdot 2H_2O$ = 14.7 g/100 ml H_2O, dissolve and 0.2 µm filter)

Protocol

Step 1.
Autoclave BD Difco AC Broth and NaCl Qs to 1 L with distilled water for 20 min.

DURATION
00:20:00

NOTES
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See formulation in guidelines.

Step 2.
After broth has cooled, add 16 ml 2 M $MgSO_4$ and 10 ml 1 M $CaCl_2$

NOTES
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(2 M $MgSO_4 \cdot 7H_2O$ = 49.3 g/100 ml H_2O, dissolve and 0.2 µm filter)
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(1 M $CaCl_2 \cdot 2H_2O$ = 14.7 g/100 ml H_2O, dissolve and 0.2 µm filter)

Step 3.

Dispense 2.0 or 4.5 ml per sterile tube

Step 4.
Inoculate sterile MPB with 1:5 (vol:vol) dilution of culture

Step 5.
Incubate at 22°C

NOTES

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Heterotrophs normally grow in less than 2 days. No growth in the broth after 1-2 weeks indicates culture is axenic.