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Formulation of MBBM (Modified Bold's Basal Medium)

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Abstract

This medium is for growing *Chlorella variabilis* NC64A, *Chlorella heliozoae* SAG 3.83, and *Coccomyxa* C-169.

Guidelines

BBM Stock Solutions

1. 25.0 gm $NaNO_3$ per liter d-H_2O
2. 2.5 gm $CaCl_2 \cdot 2H_2O$ per liter d-H_2O
3. 7.5 gm $MgSO_4 \cdot 7H_2O$ per liter d-H_2O
4. 7.5 gm K_2HPO_4 per liter d-H_2O
5. 17.5 gm KH_2PO_4 per liter d-H_2O
6. 2.5 gm NaCl per liter d-H_2O
7. 50.0 gm disodium EDTA, 31.0 gm KOH per liter d-H_2O
8. 4.98 gm $FeSO_4 \cdot 7H_2O$ per liter acidified H_2O (Acidified H_2O is 999.0 mL d-H_2O + 1.0 mL concentrated H_2SO_4)
9. 11.42 gm H_3BO_3 per liter d-H_2O
10. 8.82 gm $ZnSO_4 \cdot 7H_2O$, 1.44 gm $MnCl_2 \cdot 4H_2O$, 0.71 gm MoO_3, 1.57 gm $CuSO_4 \cdot 5H_2O$, and 0.49 gm $Co(NO_3)_2 \cdot 6H_2O$ per liter d-H_2O

Note: Stock solution 10 takes weeks for all of the salts to dissolve. Use as suspension until then.

BBM Preparation

to 950 mL of d-H_2O add:

10.0 mL of stock solutions 1, 2, 3, 4, 5 and 6
1.0 mL of stock solutions 7, 8 and 9
2.0 mL of stock solution 10

MBBM Preparation

to 950 mL of d-H_2O add:

10.0 mL of stock solutions 1, 2, 3, 4, 5 and 6
1.0 mL of stock solutions 7, 8 and 9
2.0 mL of stock solution 10
1.0 gm of bacto-peptone
5.0 gm of sucrose

Tetracycline (filter sterilized, 10 µg/mL final concentration) is added after the media is autoclaved and cool.

For MBBM plates, agar is added to 1.5% before autoclaving.

For MBBM soft agar (for titering), agar is added to 0.75% before autoclaving.