

MBBM Media Version 2

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Abstract

Van Etten, J. (n.d.). Formulation of Modified Bold's Basal Medium (MBBM). Retrieved from <http://ncv.unl.edu/vanettenlab/>

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Protocol

Add Bacto-peptone

Step 1.

Add 1.0 g bacto-peptone to a 1 L media bottle

Add sucrose

Step 2.

Add 5.0 g sucrose to the media bottle

Add Milli-Q Water

Step 3.

Bring reagents to 950 mL Milli-Q or d-H₂O

Add Agar

Step 4.

If making MBBM agar ONLY:

* For MBBM agar plates, add 1.5% agar prior to autoclaving

* For MBBM soft agar, add 0.75% agar prior to autoclaving

Add Stock Solution #1-6

Step 5.

Add 10 mL each of MBBM stock solutions #1-6 to the media bottle

*Refer to MBBM stock solutions protocol

PROTOCOL

. MBBM Stock Solutions

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Stock Solution #1

Step 5.1.

Add 25.0 g NaNO_3 per liter d- H_2O

Stock Solution #2

Step 5.2.

Add 2.5 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ per liter d- H_2O

Stock Solution #3

Step 5.3.

Add 7.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per liter d- H_2O

Stock Solution #4

Step 5.4.

Add 7.5 g K_2HPO_4 per liter d- H_2O

Stock Solution #5

Step 5.5.

Add 17.5 g KH_2PO_4 per liter d- H_2O

Stock Solution #6

Step 5.6.

Add 2.5 g NaCl per liter d- H_2O

Stock Solution #7

Step 5.7.

Add 50.0 g disodium EDTA and 31.0 g KOH per liter d- H_2O

Stock Solution #8

Step 5.8.

Add 4.98 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per liter acidified H_2O

(Acidified H₂O is 999.0 mL d-H₂O + 1.0 mL concentrated H₂SO₄)

Stock Solution #9

Step 5.9.

Add 11.42 g H₃BO₃ per liter d-H₂O

Stock Solution #10

Step 5.10.

Add per liter d-H₂O

- 8.82 g ZnSO₄*7H₂O
- 1.44 g MnCl₂*4H₂O, 0.71 g MoO₃
- 1.57 g CuSO₄*5H₂O
- 0.49 g CoNO₃*6H₂O

Autoclave

Step 5.11.

Autoclave all stock solutions at 121°C for 20 min except solution #8 and #10 (stir to dissolve)

Add Stock Solution #7-9

Step 6.

Add 1.0 mL each of stock solution #7-9 to the media bottle

*Refer to MBBM stock solutions protocol

Add Stock Solutions #7-9

Step 7.

Add 2.0 mL stock solution #10 to the media bottle

*Refer to MBBM stock solutions protocol

Autoclave

Step 8.

Autoclave at 121°C for 20 min

Add Antibiotics

Step 9.

Let media cool to room temperature and add antibiotics:

- * 700 µg/mL Ampicillin, and/or
- * 10 µg/mL Tetracycline