MBBM Media Version 3

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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/
Contact Dr. Steven Wilhelm (wilhelm@utk.edu) or Samantha Coy (srose16@vols.utk.edu) for additional information regarding this protocol.

Citation: Dr. Steven Wilhelm, Samantha Coy MBBM Media. **protocols.io**
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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-H_2O

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

CONTACT: Steven Wilhelm

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 $CaCl_2$*$2H_2O$ per liter d-H_2O

Stock Solution #3

Step 5.3.

Add 7.5 g $MgSO_4$*$7H_2O$ 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 $FeSO_4*7H_2O$ per liter acidificed H_2O

(Acidified H_2O is 999.0 mL d-H_2O + 1.0 mL concentrated H_2SO_4)

Stock Solution #9

Step 5.9.

Add 11.42 g H_3BO_3 per liter d-H_2O

Stock Solution #10

Step 5.10.

Add per liter d-H_2O

- 8.82 g $ZnSO_4*7H_2O$
- 1.44 g $MnCl_2*4H_2O$, 0.71 g MoO_3
- 1.57 g $CuSO_4*5H_2O$
- 0.49 g $CoNO_3*6H_2O$

Autoclave

Step 5.11.

Autoclave all stock solutions at 121ºC 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 121ºC 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