

# MBBM Media

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## Abstract

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## Protocol

### Step 1.

Prepare 10 stock solutions per 1 liter Milli-Q H<sub>2</sub>O each:

1. ) 25.0 g NaNO<sub>3</sub>
2. ) 2.5 g CaCl<sub>2</sub>\*2H<sub>2</sub>O
3. ) 7.5 g MgSO<sub>4</sub>\*7H<sub>2</sub>O
4. ) 7.5 g K<sub>2</sub>HPO<sub>4</sub>
5. ) 17.5 g KH<sub>2</sub>PO<sub>4</sub>
6. ) 2.5 g NaCl
7. ) 50.0 g disodium EDTA, 31.0 g KOH
8. ) 4.98 g FeSO<sub>4</sub>\*7H<sub>2</sub>O per 1 liter acidified H<sub>2</sub>O (acidified H<sub>2</sub>O: 999.0 mL Milli-Q H<sub>2</sub>O and 1.0 mL concentrated H<sub>2</sub>SO<sub>4</sub>)
9. ) 11.42 g H<sub>3</sub>BO<sub>3</sub>
10. ) 8.82 g ZnSO<sub>4</sub>\*7H<sub>2</sub>O, 1.44 g MnCl<sub>2</sub>\*4H<sub>2</sub>O, 0.71 g MoO<sub>3</sub>, 1.57 g CuSO<sub>4</sub>\*5H<sub>2</sub>O, and 0.49 g CoNO<sub>3</sub>\*6H<sub>2</sub>O

### Step 2.

Add to 950 mL Milli-Q H<sub>2</sub>O:

1. ) 1.0 g bacto-peptone
2. ) 5.0 g sucrose
3. ) 10.0 mL of stock solution 1-6
4. ) 1.0 mL of stock solutions 7-9
5. ) 2.0 mL stock solution 10

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

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

**Step 3.**

Autoclave at 121°C for 20 min

**Step 4.**

Let media cool to room temperature and add antibiotics:

\* 700 µg/mL Ampicillin, and/or

\* 10 µg/mL Tetracycline