

# HEPES MEDIUM

## UTEX MEDIUM RECIPE

### DIRECTIONS

Modification of the original recipe that changed the buffer of volvox medium to accommodate xenic cultures of LB 1662 *Glaucosphaera vacuolata*. Alkaline medium suitable for axenic and xenic cultures, especially LB 2411 *Euglena sp.* and LB 1333 *Pithophora sp.*

### For 1 Liter Total

1. To approximately 950 mL of dH<sub>2</sub>O, add each of the components in the order specified (except vitamins) while stirring continuously.
2. Adjust the pH to 8.2.
3. Bring the total volume to 1 Liter with dH<sub>2</sub>O.  
*\* For 1.5% agar medium add 15 g of agar into the flask; do not mix.*
4. Cover and autoclave medium.
5. Allow to cool and add vitamins.  
*\* For agar medium add vitamins, mix, and dispense before agar solidifies.*
6. Store at refrigerator temperature.

#	COMPONENT	AMOUNT	STOCK SOLUTION CONCENTRATION	FINAL CONCENTRATION
1	<b>Ca(NO<sub>3</sub>)<sub>4</sub>•4H<sub>2</sub>O</b> (CAS: 13477-34-4)	1 mL/L	11.8 g/100 mL dH <sub>2</sub> O	0.5 mM
2	<b>MgSO<sub>4</sub>•7H<sub>2</sub>O</b> (CAS: 10034-99-8)	1 mL/L	4 g/100 mL dH <sub>2</sub> O	0.16 mM
3	<b>Na<sub>2</sub>glycerophosphate•5H<sub>2</sub>O</b> (CAS: 13408-09-8)	1 mL/L	5 g/100 mL dH <sub>2</sub> O	0.16 mM
4	<b>KCl</b> (CAS: 7447-40-7)	1 mL/L	5 g/100 mL dH <sub>2</sub> O	0.67 mM
5	<b>HEPES buffer</b> (CAS: 7365-45-9)	0.94 g/L		3.9 mM
6	<b>P-IV Metal Solution</b>	6 mL/L	<i>See following pages for recipe.</i>	
7	<b>Vitamin B12</b>	1 mL/L	<i>See following pages for recipe.</i>	
8	<b>Biotin Vitamin Solution</b>	1 mL/L	<i>See following pages for recipe.</i>	

## DIRECTIONS

### For 1 Liter Total

1. To approximately 950 mL of dH<sub>2</sub>O, add the nutrients in the order listed while stirring continuously.

\* **Note:** The Na<sub>2</sub>EDTA should be fully dissolved before adding other components.

2. Bring total volume to 1 Liter with dH<sub>2</sub>O.

3. Store at refrigerator temperature.

#	COMPONENT	AMOUNT	STOCK SOLUTION CONCENTRATION
1	<b>Na<sub>2</sub>EDTA•2H<sub>2</sub>O</b> (CAS: 6381-92-6)	0.75 g/L	2 mM
2	<b>FeCl<sub>3</sub>•6H<sub>2</sub>O</b> (CAS: 10025-77-1)	0.097 g/L	0.36 mM
3	<b>MnCl<sub>2</sub>•4H<sub>2</sub>O</b> (CAS: 13446-34-9)	0.041 g/L	0.21 mM
4	<b>ZnCl<sub>2</sub></b> (CAS: 7646-85-7)	0.005 g/L	0.037 mM
5	<b>CoCl<sub>2</sub>•6H<sub>2</sub>O</b> (CAS: 7791-13-1)	0.002 g/L	8.4 μM
6	<b>Na<sub>2</sub>MoO<sub>4</sub>•2H<sub>2</sub>O</b> (CAS: 10102-40-6)	0.004 g/L	0.017 mM

## Vitamin B<sub>12</sub> Solution Component

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### DIRECTIONS

#### For 200 mL Total

1. Prepare 200 mL of HEPES buffer.
2. Adjust the pH to 7.8.
3. Add Vitamin B<sub>12</sub> and wait until fully dissolved.
4. Sterilize by 0.45 µM Millipore filter.
5. Store in the dark at freezer temperature.

#	COMPONENT	AMOUNT	STOCK SOLUTION CONCENTRATION
1	<b>HEPES buffer pH 7.8</b> (CAS: 7365-45-9)	2.4 g/200 mL dH <sub>2</sub> O	50 mM
2	<b>Vitamin B<sub>12</sub> (cyanocobalamin)</b> (CAS: 68-19-9)	0.027 g/200 mL dH <sub>2</sub> O	0.1 mM

## Biotin Vitamin Solution Component

### DIRECTIONS

#### For 200 mL Total

1. Prepare 200 mL of HEPES buffer.
2. Adjust the pH to 7.8.
3. Add biotin (0.1 mM) and wait until fully dissolved.
4. Sterilize by 0.45 µM Millipore filter.
5. Store in the dark at freezer temperature.

#	COMPONENT	AMOUNT	STOCK SOLUTION CONCENTRATION
1	<b>HEPES buffer pH 7.8</b> (CAS: 7365-45-9)	2.4 g/200 mL dH <sub>2</sub> O	50 mM
2	<b>Biotin</b> (CAS: 58-85-5)	0.005 g/200 mL dH <sub>2</sub> O	~0.1 mM