HEPES 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 L Total
1. To approximately 950 mL of dH_2O, 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 L with dH_2O.
*For 1.5% agar medium add 15 g of agar into the flask; do not mix.
4. Cover and autoclave medium.
5. When cooled 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	$Ca(NO_3)_2 \cdot 4H_2O$ (Sigma C 5676)	1 mL/L	11.8 g/100 mL dH20	0.5 mM
2	$MgSO_4 \cdot 7H_2O$ (Sigma 230391)	1 mL/L	4 g/100 mL dH20	0.16 mM
3	Na_2glycerophosphate$\cdot 5H_2O$ (Sigma G 6501)	1 mL/L	5 g/100 mL dH20	0.16 mM
4	KCl (Fisher P 217)	1 mL/L	5 g/100 mL dH20	0.67 mM
5	HEPES buffer (Sigma H-3375)	0.94 g/L		3.9 mM
6	P-IV Metal Solution	6 mL/L		
7	Vitamin B_{12}	1 mL/L		
8	Biotin Vitamin Solution	1 mL/L		