P49 MEDIUM

UTEX MEDIUM RECIPE

DIRECTIONS

Suitable for volvocales, especially *Chlamydomonas* and *Pandorina*, which may benefit primarily from the ammonium component, modified volvox medium.

For 1 Liter Total

1. To approximately 900 mL of dH_2O, add each of the components in the order specified (except vitamins) while stirring continuously.
2. Adjust the pH to 7.0.
3. Bring the total volume to 1 Liter 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. 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	**$MgSO_4 \cdot 7H_2O$** (CAS: 10034-99-8)	1 mL/L	11.8 g/100 mL dH_2O	0.5 mM
2	**Na_2glycerophosphate$\cdot 5H_2O$** (CAS: 13408-09-8)	1 mL/L	4 g/100 mL dH_2O	0.16 mM
3	**KCl** (CAS: 7447-40-7)	1 mL/L	5 g/100 mL dH_2O	0.16 mM
4	**Glycylglycine** (CAS: 556-50-3)	1 mL/L	5 g/100 mL dH_2O	0.67 mM
5	**P-IV Metal Solution**	6 mL/L	*See following pages for recipe.*	
6	**NH_4NO_3** (CAS: 6484-52-2)	10 mL/L	1 g/100 mL dH_2O	1.2 mM
7	**$CaCl_2 \cdot 2H_2O$** (CAS: 10035-04-8)	10 mL/L	0.74 g/100 mL dH_2O	0.5 mM
8	**Yeast Extract** (CAS: 8013-01-2)	0.2 g/L		
9	**Tryptone** (CAS: 91079-40-2)	0.4 g/L		
10	**Vitamin B12**	1 mL/L	*See following pages for recipe.*	
11	**Biotin Vitamin Solution**	1 mL/L	*See following pages for recipe.*	
12	**Thiamine Vitamin Solution**	1 mL/L	*See following pages for recipe.*	

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P-IV Metal Solution Component

DIRECTIONS

For 1 Liter Total

1. To approximately 950 mL of dH_2O, add the nutrients in the order listed while stirring continuously.
   * ***Note:*** The Na_2EDTA should be fully dissolved before adding other components.
2. Bring total volume to 1 Liter with dH_2O.
3. Store at refrigerator temperature.

#	COMPONENT	AMOUNT	STOCK SOLUTION CONCENTRATION
1	**$Na_2EDTA•2H_2O$** (CAS: 6381-92-6)	0.75 g/L	2 mM
2	**$FeCl_3•6H_2O$** (CAS: 10025-77-1)	0.097 g/L	0.36 mM
3	**$MnCl_2•4H_2O$** (CAS: 13446-34-9)	0.041 g/L	0.21 mM
4	**$ZnCl_2$** (CAS: 7646-85-7)	0.005 g/L	0.037 mM
5	**$CoCl_2•6H_2O$** (CAS: 7791-13-1)	0.002 g/L	8.4 µM
6	**$Na_2MoO_4•2H_2O$** (CAS: 10102-40-6)	0.004 g/L	0.017 mM

Vitamin B_{12} Solution Component

DIRECTIONS

For 200 mL Total

1. Prepare 200 mL of HEPES buffer.
2. Adjust the pH to 7.8.
3. Add Vitamin B_{12} 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	**HEPES buffer pH 7.8** (CAS: 7365-45-9)	2.4 g/200 mL dH_2O	50 mM
2	**Vitamin B_{12} (cyanocobalamin)** (CAS: 68-19-9)	0.027 g/200 mL dH_2O	0.1 mM

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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	**HEPES buffer pH 7.8** (CAS: 7365-45-9)	2.4 g/200 mL dH_2O	50 mM
2	**Biotin** (CAS: 58-85-5)	0.005 g/200 mL dH_2O	~0.1 mM

Thiamine Vitamin Solution Component

DIRECTIONS

For 200 mL Total

1. Prepare 200 mL of HEPES buffer.
2. Adjust the pH to 7.8.
3. Add Thiamine (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	**HEPES buffer pH 7.8** (CAS: 7365-45-9)	2.4 g/200 mL dH_2O	50 mM
2	**Thiamine** (CAS: 67-03-8)	0.067 g/200 mL dH_2O	~1 mM

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