

DYIII MEDIUM

UTEX MEDIUM RECIPE

DIRECTIONS

Modification of Sandgren's recipe. Suitable for xenic cultures of freshwater chrysophytes.

For 1 Liter Total

- To approximately 950 mL of dH₂O, add each of the components in the order specified (except vitamins) while stirring continuously.
- Adjust the pH to 6.7.
- Bring the total volume to 1 Liter with dH₂O.
 - * For 1.5% agar medium add 15 g of agar into the flask; do not mix.
- Cover and autoclave medium.
- Allow to cool and add vitamins.
 - * For agar medium add vitamins, mix, and dispense before agar solidifies.
- Store at refrigerator temperature.

#	COMPONENT	AMOUNT	STOCK SOLUTION CONCENTRATION	FINAL CONCENTRATION
1	MES (CAS: 7365-45-9)	1.95 g/L		10 mM
2	MWC Metal Solution	10 mL/L		
3	CaCl₂•2H₂O (CAS: 10035-04-8)	1 mL/L	2 g/100 mL dH ₂ O	0.14 mM
4	MgSO₄•7H₂O (CAS: 10034-99-8)	1 mL/L	7.4 g/100 mL dH ₂ O	0.3 mM
5	Na₂glycerophosphate•5H₂O (CAS: 13408-09-8)	1 mL/L	1 g/100 mL dH ₂ O	0.03 mM
6	NaNO₃ (CAS: 7631-99-4)	1 mL/L	2 g/100 mL dH ₂ O	0.24 mM
7	Na₂SiO₃•9H₂O (CAS: 13517-24-3)	1 mL/L	1.5 g/100 mL dH ₂ O	0.05 mM
8	NH₄NO₃ (CAS: 6484-52-2)	1 mL/L	1 g/100 mL dH ₂ O	0.12 mM
9	KCl (CAS: 7447-40-7)	1 mL/L	1 g/100 mL dH ₂ O	0.13 mM
10	Vitamin B12	1 mL/L	<i>See following pages for recipe.</i>	
11	Biotin Vitamin Solution	1 mL/L	<i>See following pages for recipe.</i>	
12	Thiamine Vitamin Solution	1 mL/L	<i>See following pages for recipe.</i>	

MWC Metal Solution Component

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DIRECTIONS

For 1 Liter Total

1. To approximately 950 mL of dH₂O, add the nutrients in the order listed while stirring continuously.

* **Note:** Each component should be fully dissolved before adding the next.

2. Bring total volume to 1 Liter with dH₂O.

3. Store at refrigerator temperature.

#	COMPONENT	AMOUNT	STOCK SOLUTION CONCENTRATION
1	Na₂EDTA•2H₂O (CAS: 6381-92-6)	0.44 g/L	0.012 mM
2	H₃BO₃ (CAS: 10043-35-3)	0.097 g/L	0.016 mM
3	FeCl₃•6H₂O (CAS: 10025-77-1)	0.315 g/L	0.01 mM
4	MnCl₂•4H₂O (CAS: 13446-34-9)	0.018 g/L	0.001 mM
5	ZnSO₄•7H₂O (CAS: 7446-20-0)	0.002 g/L	0.07 µM
6	CoCl₂•6H₂O (CAS: 7791-13-1)	0.001 g/L	0.042 µM
7	Na₂MoO₄•2H₂O (CAS: 10102-40-6)	0.0006 g/L	0.025 µM

Vitamin B₁₂ Solution Component

DIRECTIONS

For 200 mL Total

1. Prepare 200 mL of HEPES buffer.

2. Adjust the pH to 7.8.

3. Add Vitamin B₁₂ 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 ₂ O	50 mM
2	Vitamin B₁₂ (cyanocobalamin) (CAS: 68-19-9)	0.027 g/200 mL dH ₂ O	0.1 mM

Biotin Vitamin 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 biotin (0.1 mM) and wait until fully dissolved.
4. Sterilize by 0.45 μ M 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 ₂ O	50 mM
2	Biotin (CAS: 58-85-5)	0.005 g/200 mL dH ₂ O	~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 μ M 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 ₂ O	50 mM
2	Thiamine (CAS: 67-03-8)	0.067 g/200 mL dH ₂ O	~1 mM