

DY-III Medium

(Lehman 1976)

This artificial freshwater medium was developed for physiological studies of *Dinobryon*, especially with regard to phosphorus and potassium. The MES buffer and the iron wire dissolved in HCl result in a very low pH that must be adjusted.

To prepare, begin with 950 mL of dH₂O, add the following components as well as 1 mL each of the trace metal and vitamin solutions. Bring the final volume to 1 liter using dH₂O. Adjust pH to 6.8 with NaOH. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
CaCl ₂ • 2H ₂ O	75.00 g L ⁻¹ dH ₂ O	1 mL	5.10 x 10 ⁻⁴ M
Na ₂ SiO ₃ • 9 H ₂ O	15.00 g L ⁻¹ dH ₂ O	1 mL	5.28 x 10 ⁻⁵ M
MgSO ₄ • 7H ₂ O	50.00 g L ⁻¹ dH ₂ O	1 mL	2.03 x 10 ⁻⁴ M
MES buffer	---	200 mg	1.02 x 10 ⁻³ M
NH ₄ NO ₃	5.00 g L ⁻¹ dH ₂ O	1 mL	6.25 x 10 ⁻⁵ M
NaNO ₃	20.00 g L ⁻¹ dH ₂ O	1 mL	2.35 x 10 ⁻⁴ M
KCl	3.00 g L ⁻¹ dH ₂ O	1 mL	4.02 x 10 ⁻⁵ M
Na ₂ b-glycerophosphate	10.00 g L ⁻¹ dH ₂ O	1 mL	4.63 x 10 ⁻⁵ M
H ₃ BO ₃	4.58 g L ⁻¹ dH ₂ O	1 mL	7.41 x 10 ⁻⁵ M
trace metals solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	1 mL	---

Trace Metals Solution

First prepare the primary stock solutions. In the original recipe, iron wire is dissolved in a small amount of concentrated HCl and added to dH₂O. Alternatively, a ferric chloride

(FeCl₃ 6 H₂O) primary stock solution can be prepared by adding 3.389 g to 1 liter dH₂O, and this will provide the same amount of iron; the additional chloride (5 x 10⁻⁶ M in final medium) is probably insignificant. To prepare the final trace metal solution, add 1 mL of each primary stock to 950 mL dH₂O and then bring the final volume to 1 liter with dH₂O.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
Na ₂ EDTA (anhydrous)	8.000 g L ⁻¹ dH ₂ O	1 mL	2.74 x 10 ⁻⁵ M
Fe (iron wire)	0.700 g L ⁻¹ dH ₂ O	1 mL	1.25 x 10 ⁻⁵ M
MnCl ₂ • 4H ₂ O	0.720 g L ⁻¹ dH ₂ O	1 mL	3.54 x 10 ⁻⁶ M
ZnSO ₄ • 7H ₂ O	0.176 g L ⁻¹ dH ₂ O	1 mL	6.12 x 10 ⁻⁷ M
Na ₂ MoO ₄ • 2H ₂ O	0.050 g L ⁻¹ dH ₂ O	1 mL	2.07 x 10 ⁻⁷ M
CoCl ₂ • 6H ₂ O	0.032 g L ⁻¹ dH ₂ O	1 mL	1.34 x 10 ⁻⁷ M

Vitamin Solution

To prepare, begin with 950 mL of dH₂O, add the components and bring final volume to 1 liter with dH₂O. Filter sterilize, store in refrigerator or freezer.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine • HCl (vit. B ₁)	---	200 mg	5.93 x 10 ⁻⁷ M
biotin (vit. H)	0.5 g L ⁻¹ dH ₂ O	1 mL	2.05 x 10 ⁻⁹ M
cyanocobalamin (vit. B ₁₂)	0.5 g L ⁻¹ dH ₂ O	1 mL	3.69 x 10 ⁻¹⁰ M

Lehman, J.T. 1976. Ecological and nutritional studies on *Dinobryon* Ehrenb.: Seasonal periodicity and the phosphate toxicity problem. *Limnol. Oceanog.* **21**: 646-658.