Cyanophora DY-V Medium

(NCMA)

Cyanophora medium is simply DY-V medium plus additions of organics and soil water extract. DY-V is an artificial freshwater medium that was developed for chromophytes, especially chrysophytes and synurophytes. DY-V is derived from DY-III medium (Lehman 1976), although first modified as DY-IV by adding more trace metals (Keller and Andersen, in Andersen et al. 1997). DY-V was further modified by increasing the nitrogen and phosphorus concentrations. Its primary disadvantage is that fungi grow very well in the medium, probably due to the combination of glycerophosphate and ammonium, and therefore careful sterile technique is required. The MES buffer is pH adjusted to outside its buffering range, but removal of the MES hinders growth. The soil water extract is prepared by autoclaving 20 g of soil (from virgin prairie of North Dakota) in 100 mL of distilled water.

To prepare, begin with 950 mL of dH_20, add the following components and bring the final volume to 1 liter using dH_2O. Adjust pH to 6.8 with NaOH. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
MES	---	200 mg	1.02×10^{-3} M
$MgSO_4 \cdot 7H_2O$	50 g/L dH_2O	1 mL	2.03×10^{-4} M
KCl	3 g/L dH_2O	1 mL	4.02×10^{-5} M
NH_4Cl	2.68 g/L dH_2O	1 mL	5.01×10^{-5} M
$NaNO_3$	20 g/L dH_2O	1 mL	2.35×10^{-4} M
Na_2 b-glycerophosphate	2.16 g/L dH_2O	1 mL	1.00×10^{-5} M
H_3BO_3	0.8 g/L dH_2O	1 mL	1.29×10^{-5} M
$Na_2SiO_3 \cdot 9\ H_2O$	14 g/L dH_2O	1 mL	4.93×10^{-5} M
$CaCl_2$	75 g/L dH_2O	1 mL	6.76×10^{-4} M
soil water extract	---	1 mL	---
organics solution	(see recipe)	1 mL	---
trace element solution	(see recipe)	1 mL	---
f/2 vitamin solution	(see recipe)	0.5 mL	---

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Organics Solution

To prepare, dissolve the following components in 900 mL of dH_2O and bring the final volume to 1 liter. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
Bacto-peptone	---	20.0 g	---
Malt extract	---	50.0 g	---

Trace Element Solution

To prepare, separately dissolve the following components in 100 mL of dH_2O. Combine the six solutions (= 600 mL) and bring the final volume to 1 liter. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
$Na_2EDTA \cdot 2H_2O$	---	8.0 g	2.15×10^{-5} M
$FeCl_3 \cdot 6\ H_2O$	---	1.0 g	3.70×10^{-6} M
$MnCl_2 \cdot 4H_2O$	---	200 mg	1.01×10^{-6} M
$ZnSO_4 \cdot 7H_2O$	---	40 mg	1.39×10^{-7} M
$CoCl_2 \cdot 6H_2O$	8.0 g L^{-1} dH_2O	1 mL	3.36×10^{-8} M
$Na_2MoO_4 \cdot 2H_2O$	20.0 g L^{-1} dH_2O	1 mL	8.27×10^{-8} M
$Na_3VO_4 \cdot 10H_2O$	2.0 g L^{-1} dH_2O	1 mL	5.49×10^{-9} M
H_2SeO_3	4 g L^{-1} dH_2O	1 mL	2.31×10^{-8} M

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f/2 Vitamin Solution

(Guillard & Ryther 1962, Guillard 1975)

First, prepare primary stock solutions. To prepare final vitamin solution, begin with 950 mL of dH_2O, dissolve the thiamine, add 1 mL of the primary stocks and bring final volume to 1 liter with dH_2O. Filter sterilize. Store in refrigerator or freezer.

Component	PrimaryStock Solution	Quantity	Molar Concentration in Final Medium
thiamine · HCl (vit. B_1)	---	200 mg	2.96×10^{-7} M
biotin (vit. H)	1.0 g/L dH_2O	1 mL	2.05×10^{-9} M
cyanocobalamin (vit. B_{12})	1.0 g/L dH_2O	1 mL	3.69×10^{-10} M

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