DY-V Medium and modification as well as derivatives

(NCMA)

This artificial freshwater medium was developed for chromophytes, especially chrysophytes and synurophytes. It is derived from DY-III medium developed by Lehman (1976). The medium was first modified as DY-IV by adding more trace metals (Keller and Andersen, in Andersen et al. 1997) and 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. ***Note: Recipe below lists the phosphate source currently used within the NCMA collection. The table on page 3 of this recipe lists the historically used phosphate source and its concentration.**

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
$NaH_2PO_4 \cdot H_2O$	5.00 g L^{-1} dH_2O	1 mL	3.62×10^{-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
$CaCl_2 \cdot 2 H_2O$	75 g/L dH_2O	1 mL	5.10×10^{-4} M
trace element solution	(see recipe)	1 mL	---
f/2 vitamin solution	(see recipe)	0.5 mL	---

Trace Element Solution

To prepare, begin with 900 mL of dH_2O and dissolve the EDTA. Next, dissolve each compound and bring the final volume to 1 liter. Autoclave. If precipitation occurs during storage, it can usually be re-dissolved by heating or by adding a small amount of sodium hydroxide to make the solution slightly more basic.

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

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 the amounts of the primary stocks as indicated in the quantity column below, and bring final volume to 1 liter with dH_2O. At the NCMA we autoclave to sterilize. Store in refrigerator or freezer.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine · HCl (vit. B_1)	---	200 mg	2.96×10^{-7} M
biotin (vit. H)	0.1 g/L dH_2O	10mL	2.05×10^{-9} M
cyanocobalamin (vit. B_{12})	1.0 g/L dH_2O	1 mL	3.69×10^{-10} M

Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. pp 26-60. *In* Smith W.L. and Chanley M.H (Eds.) *Culture of Marine Invertebrate Animals*. Plenum Press, New York, USA.

Guillard, R.R.L. and Ryther, J.H. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Can. J. Microbiol.* **8:** 229-239.

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

***NCMA began utilizing f/2 phosphate (labeled as DYV-m) within the collection to reduce bacterial growth in our non-axenic cultures. DY-V historically was made using Na_2 b-glycerophosphate. The concentration of this previously used compound is listed in the table below.**

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
Na_2 b-glycerophosphate	2.16 g/L dH_2O	1 mL	1.00 x 10^{-5} M

DY-V derivatives

These can be made using DY-V or DY-Vm media.

DY-V + Rice: For heterotrophic organisms. The rice becomes food for bacteria that a eukaryote may eat, or it can supply organics as a source of nutrition. Add a grain of rice to a tube of DY-V medium and then autoclave. This softens the rice and allows for maximum dissolution of the rice grain. If too much organics are released using this method, rice can be autoclaved separately and then added aseptically to sterile medium.

DY-V +Soil: for organisms that find the addition a biphasic medium beneficial. Before autoclaving, add a pinch of soil to the test tube, and then autoclave. The soil is best from areas that have not experienced the use of pesticides or herbicides.