Culture Collection
CCCryo
of Cryophilic Algae

Fraunhofer
IBMT

TAP-Medium (Tris-Acetate-Phosphate)

Lit.: Andersen, R.A. (ed.) (2005): Algal culturing techniques, 578pp, Elsevier Academic Press, London.
Gorman, D.S. & Levine, R.P. (1965): Cytochrome f and plastocyanin: their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardii*. - *Proc. Natl. Acad. Sci. U. S. A.* 54: 1665-1669.
Harris, E.H. (1989): The *Chlamydomonas* sourcebook: a comprehensive guide to biology and laboratory use. Academic Press, San Diego, 780pp.
Sueoka, N. (1960): Mitotic replication of deoxyribonucleic acid in *Chlamydomonas reinhardi*. - *Proc. Natl. Acad. Sci. U. S. A.* 46(1): 83-91.

TAP-Medium can be used for those algae which use NH_4^+ instead of NO_3^- as a nitrogen source. Such algae lack the nitrate reductase which would enable them to reduce nitrate via nitrite to ammonium.

For 1000 mL final culture medium add the following quantities (Volume) of stock solutions (SL) prepared at the given concentrations to 850 mL dd-H_2O. Add **one component after the other until each one has completely mixed** and finally fill up to 1000 mL.
All stock solutions can be stored unsterilised at 4 °C.

Stock Solution (SL)	Volume	Component	Concentration in SL	Conc. in final Medium
Tris base	2.42 g	$H_2NC(CH_2OH)_3$ Tris(hydroxymethyl)-aminomethan		$2.00 \cdot 10^{-2}$ M
TAP-salts (Beijerinck salts)	25 mL	NH_4Cl	15 g · L^{-1}	$7.00 \cdot 10^{-3}$ M
		$MgSO_4 \cdot 7H_2O$	4 g · L^{-1}	$8.30 \cdot 10^{-4}$ M
		$CaCl_2 \cdot 2H_2O$	2 g · L^{-1}	$4.50 \cdot 10^{-4}$ M
Phophate solution	1 mL	K_2HPO_4	28.8 g · 100 mL^{-1}	$1.65 \cdot 10^{-3}$ M
		KH_2PO_4	14.4 g · 100 mL^{-1}	$1.05 \cdot 10^{-3}$ M
Trace elements solution (Hutner trace elements)	1 mL	$Na_2EDTA \cdot 2H_2O$	5.00 g · 100 mL^{-1}	$1.34 \cdot 10^{-4}$ M
		$ZnSO_4 \cdot 7H_2O$	2.20 g · 100 mL^{-1}	$1.36 \cdot 10^{-4}$ M
		H_3BO_3	1.14 g · 100 mL^{-1}	$1.84 \cdot 10^{-4}$ M
		$MnCl_2 \cdot 4H_2O$	0.50 g · 100 mL^{-1}	$4.00 \cdot 10^{-5}$ M
		$FeSO_4 \cdot 7H_2O$	0.50 g · 100 mL^{-1}	$3.29 \cdot 10^{-5}$ M
		$CoCl_2 \cdot 6H_2O$	0.16 g · 100 mL^{-1}	$1.23 \cdot 10^{-5}$ M
		$CuSO_4 \cdot 5H_2O$	0.16 g · 100 mL^{-1}	$1.00 \cdot 10^{-5}$ M
		$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.11 g · 100 mL^{-1}	$9.28 \cdot 10^{-7}$ M
Acetic acid, conc.	1 mL	CH_3COOH		

First dissolve $Na_2EDTA \cdot 2H_2O$ in 100 mL dd-H_2O by heating to 60-80 °C, then adjust pH with KOH to 5.0. Add all trace elements separately and check the pH value constantly. The pH value should not increase above 6.8, otherwise $MnSO_4$ may precipitate. Let the solution stand at 4 °C; when the colour changes from orange to red after approx. 2 weeks, filter it, split it, and store at -20 °C in teflon or polycarbonate containers (do not use glass containers for trace elements as these tend to adsorb to the glass surface). After addition of acetic acid the pH should range at about 7.0.

Adjust medium to final pH of 6.0 or as desired with acetic acid and autoclave at 121 °C for 20 min.

For stock cultures on agar slants add 1.0-1.3 % Agar (e.g. purified high strength, 1000 g · cm^{-2}) to prepared medium before autoclaving.