

## TAP Medium (Tris-Acetate-Phosphate Medium)

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TAP-Medium can be used for those algae which metabolise  $\text{NH}_4^+$  instead of  $\text{NO}_3^-$  as a nitrogen source. Such algae often 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- $\text{H}_2\text{O}$ . 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	$\text{H}_2\text{NC}(\text{CH}_2\text{OH})_3$ Tris(hydroxymethyl)-aminomethan		$2.00 \cdot 10^{-2}$ M
TAP-salts (Beijerinck salts)	25 mL	$\text{NH}_4\text{Cl}$ $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	$15 \text{ g} \cdot \text{L}^{-1}$ $4 \text{ g} \cdot \text{L}^{-1}$ $2 \text{ g} \cdot \text{L}^{-1}$	$7.00 \cdot 10^{-3}$ M $8.30 \cdot 10^{-4}$ M $4.50 \cdot 10^{-4}$ M
Phosphate solution	1 mL	$\text{K}_2\text{HPO}_4$ $\text{KH}_2\text{PO}_4$	$28.8 \text{ g} \cdot 100 \text{ mL}^{-1}$ $14.4 \text{ g} \cdot 100 \text{ mL}^{-1}$	$1.65 \cdot 10^{-3}$ M $1.05 \cdot 10^{-3}$ M
Trace elements solution (Hutner trace elements)	1 mL	$\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ (Titriplex III) $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ $\text{H}_3\text{BO}_3$ $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ $(\text{NH}_4)_6\text{MoO}_3$	$5.00 \text{ g} \cdot 100 \text{ mL}^{-1}$ $2.20 \text{ g} \cdot 100 \text{ mL}^{-1}$ $1.14 \text{ g} \cdot 100 \text{ mL}^{-1}$ $0.50 \text{ g} \cdot 100 \text{ mL}^{-1}$ $0.50 \text{ g} \cdot 100 \text{ mL}^{-1}$ $0.16 \text{ g} \cdot 100 \text{ mL}^{-1}$ $0.16 \text{ g} \cdot 100 \text{ mL}^{-1}$ $0.11 \text{ g} \cdot 100 \text{ mL}^{-1}$	$1.34 \cdot 10^{-4}$ M $1.36 \cdot 10^{-4}$ M $1.84 \cdot 10^{-4}$ M $4.00 \cdot 10^{-5}$ M $3.29 \cdot 10^{-5}$ M $1.23 \cdot 10^{-5}$ M $1.00 \cdot 10^{-5}$ M $4.44 \cdot 10^{-6}$ M
Acetic acid, conc.	1 mL	$\text{CH}_3\text{COOH}$		

First dissolve  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$  in 100 mL distilled water 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  $\text{MnSO}_4$  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,  $1000 \text{ g} \cdot \text{cm}^{-2}$ ) to prepared medium before autoclaving.