

Chlamydomonas Culture Medium (modified recipe from Togasaki, re-typed by Janette Kropat on 27th April 2007)

TAP medium:

Per 1000 mL: 10 mL 5 x Beijerincks or 50 ml 1 x Beijerincks
 8.33 mL phosphate solution
 10 mL Tris-Acetate stock
 1 mL Trace Elements stock

Minimal medium:

Per 1000 mL: 10 mL 5 x Beijerincks or 50 ml 1 x Beijerincks
 8.33 mL phosphate solution
 10 mL 2 M Tris base
 1 mL Trace Elements stock solution
 About 1.1 mL concentrated HCl to adjust pH to 6.8-7.2_[JK1]

Stock Solutions (molarities indicated are the final concentrations in TAP)

5 x Beijerinck's Solution:

	<u>1000mL</u>	<u>Concentration</u>
NH ₄ Cl	40 g	7.5 mM
CaCl ₂ • 2H ₂ O	5 g	0.34 mM
MgSO ₄ • 2H ₂ O	10 g	0.4 mM

- Dissolve CaCl₂ in 300 mL H₂O. Dissolve NH₄Cl and MgSO₄ in 500 mL, mix both solutions and add H₂O to 1000 mL. This takes some time to go into solution.
- If low sulfate medium is desired, replace MgSO₄ • 2H₂O with 8.15 g MgCl₂ • 6H₂O.
- For anhydrous MgSO₄ measure 4.82 g.
- Use 10 mL stock per 1000 mL of medium.

Note: The original recipe calls for 1X Beijerinck's solution so you can make it at the concentration that is convenient for you and adjust volumes appropriately.

Phosphate Solution:

	<u>1000mL</u>	<u>Concentration of phosphate</u>
K ₂ HPO ₄ (anhydrous)	14.34 g	0.68 mM
KH ₂ PO ₄ (anhydrous)	7.26 g	0.45 mM

- If K₂HPO₄ • 3H₂O is used, use 18.6 g of trihydrate instead of anhydrous.
- If metal free phosphate is desired weigh appropriate amount of KH₂PO₄ to get phosphate concentration equal to 0.136 M. Then add metal free KOH to adjust pH to 7.1 (per 1000 mL: 18.5 g KH₂PO₄ and about 28 mL 20% KOH).
- Use 8.33 mL stock per 1000 mL medium.
- original recipe was with 11.62 g Na₂HPO₄ (anhydrous) instead of K₂HPO₄ per liter of solution.

Tris-Acetate Stock Solution:

	<u>1000mL</u>	<u>Concentration</u>
Tris base	242 g	20 mM
Glacial Acetic Acid	100 mL	17 mM

- Use 10 mL per 1000 mL medium.

Trace Elements Solution (Hutner 1946, J. Bact. 52: 213):

	<u>1000mL</u>
EDTA-Na ₂	50.0 g
H ₃ BO ₃ (boric acid)	11.14 g
ZnSO ₄ • 7H ₂ O	22.0 g
MnCl ₂ • 4H ₂ O	5.1 g
FeSO ₄ • 7H ₂ O	5.0 g
CoCl ₂ • 6H ₂ O	1.6 g
CuSO ₄ • 5H ₂ O	1.6 g
(NH ₄) ₆ Mo ₇ O ₂₄ • 4H ₂ O	1.1 g

- Add elements one at a time (except EDTA) to 550 mL milliQ H₂O in a 1L Erlenmeyer flask. Heat up solution to approximately 70 °C.
 - In a different beaker, add EDTA to 250 mL milliQ H₂O and heat until dissolved.
 - Add EDTA solution to salt solution (not vice versa) and bring the combined solution to a boil.
 - Let solution cool down and maintain temperature at 70 – 75 °C.
 - Adjust pH to 6.5 – 6.8 with 20% KOH. Be careful: do not let the temperature fall below 70 °C or let the pH go over 6.8 otherwise you have to start all over again!
 - Dilute to 1000 mL.
 - Use a cotton plug to cover flask (not parafilm!) and let stand for two weeks until the color changes from green to purple. Stir solution to help it along.
 - Filter out red-brown precipitate and store in refrigerator. Note if there is a precipitate, then you don't know the real concentrations of elements in your solution.
 - Use 1 mL per 1000 mL medium.
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- For a trace element solution lacking a specific metal, leave out the appropriate metal. The solution will have a different coloration and only a little or no precipitate. Copper free trace elements solution for example will be reddish instead of purple.