Culture Conditions for Chlamydomonas reinhardi

A. Culture Media

Stock Solutions (molarities indicated are the final concentrations in tris-acetate-phosphate medium)

Beijerinck's Solution:

NH_4Cl	8 g	7.5×10^{-3} M
$CaCl_2.2H_2O$	1 g	3.4×10^{-4} M
$MgSO_4.7H_2O$	2 g	4.0×10^{-4} M
water to 1000 ml		

Phosphate Buffer:

~~Na_2HPO_4 (anhydrous)~~	~~11.66 g~~	~~6.8×10^{-4} M~~
KH_2PO_4	7.26 g	4.5×10^{-4} M
water to 1000 ml		

or

Tris-Acetate Solution:

Trizma Base (Sigma)	121 g	2.0×10^{-2} M
Glacial acetic acid	50 ml	1.7×10^{-2} M
water to 500 ml		

Hutner's Trace Elements Solutions (J. Bacteriol., 52, 213 (1946)

Ethylenediamine tetraacetic acid	50.0 g
$ZnSO_4.7H_2O$	22.0 g
H_3BO_3	11.4 g
$MnCl_2.4H_2O$	5.1 g
$FeSO_4.7H_2O$	5.0 g
$CoCl_2.6H_2O$	1.6 g
$CuSO_4.5H_2O$	1.6 g
$(NH_4)_6Mo_7O_{24}.4H_2O$	1.1 g

Boil in 750 ml distilled water, cool slightly and bring to pH 6.5-6.8 with KOH (do not use NaOH). The clear solution is diluted to 1000 ml with distilled water and should have a green color which changes to purple on standing. Stable for at least one year.

Media (using stock solutions described above)

Tris-Acetate-Phosphate Medium (pH 7.3):

	1l	3l
Beijerinck's	50	150 ml
Phosphate	8.33	25 ml
Trace	1	3 ml
Tris-Acetate	10	30 ml
H_2O	931	2.79l
water to 3000 ml		

CHLAMYDOMONAS CULTURE MEDIA

A. SOLUTIONS

1. Phosphate Buffer (2x)

K_2HPO_4	14.34 g
KH_2PO_4	7.26 g
distilled water	1000 ml

2. Beijerinck's Solution (2x)

NH_4Cl	8 g
$CaCl_2 \cdot 2H_2O$	1 g
$MgSO_4 \cdot 7H_2O$	2 g

3. Trace Elements (after Hutner, S. H., 1950, Proc. Amer. Phil. Soc. 94: 152-170)

Ethylene diamine (dinitrilo) tetra-acetic acid disodium salt	50.0 g	
$ZnSO_4 \cdot 7H_2O$ FW288	22.0 g	16.93 gAc. $ZnAc_2$ FW 219
H_3BO_3	11.4 g	
$MnCl_2 4H_2O$	5.06g	
$FeSO_4 \cdot 7H_2O$ 278	4.99g	3.58 gCl. $FeCl_2$ FW199.
$CoCl_2 \cdot 6H_2O$	1.61g	
$CuSO_4 \cdot 5H_2O$ 250	1.57g	1.26 gAc. $CuAc_2$ FW 200.
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1.10g	

Directions: Add all but the EDTA to 550 ml distilled water, dissolving them one at a time. Put EDTA into 250 ml distilled water, heat until completely dissolved. Bring the first beaker to 70°C, add the EDTA solution. Keeping the total solution at or above 70°C, bring to pH 6.5-6.8 by adding 20% KOH solution (put 20 gm KOH in 100 ml H_2O). (Do not use NaOH, or KOH pellets undissolved. Do not make more alkaline -- it cannot be brought back with acid). Dilute to 1000 ml. Let stand in flask with cotton top until the solution turns purple (at room temperature). Filter out red-brown precipitate. Keep in refrigerator.

B. MEDIA

1. TAP

Trizma 121	2.42	g
glacial acetic acid	1.0	ml
1 M KH_2PO_4, pH 7.0	1.0	ml
Beijerinck's solution (2x)	50	ml
Trace solution	1	ml
distilled water	950	ml

2. MINIMAL

			plates mm	MYA
Beijerinck's solution (2x)	50	ml	35	35
Phosphate Buffer (2x)	50	ml	35	35
Trace Solution	1	ml	.7	.7
Distilled water	900	ml	630	630
			700ml	2.3g Difco
			10.5g agar	2.3g E

3. SUPPLEMENTED MEDIA

a. acetate: minimal plus 2g NaAcetate.$3H_2O$ per litre

b. yeast acetate: minimal plus ~~2g~~ 3.3 Sodium acetate and 4g yeast extract

4. NOTES

a. for liquid media use as given above. We routinely use 300 ml in a 500 ml flask.

b. for plates use 1.5% agar, or 15g per litre. Allow 30 ml per plate.

c. for agar slants use 2% agar, 20g per litre.

C. CARE OF CULTURES

1. All stocks are kept in yeast acetate agar slants with screw tops. After innoculation, let them grow up for a week at 500 ft-candles (less for photosynthetic mutants) with the tops loose, then tighten tops and keep in dim light.

2. Mutants currently in use are also kept on several Petrie dishes, yeast extract for wild type and most mutants, TAP for photosynthetic mutants. The plates are transfered every week, or every 3-5 days for photosynthetic mutants. Light is 500 ft-candles for most, 50 or so for the photosynthetics.

3. For biochemical work, flasks of liquid media (usually TAP) are innoculated from plates (the cells first being suspended in a small quantity of sterile water). The shaker has a light level of about 500 ft-candles; for photosynthetic mutants a paper towel around the flask reduces the light by at least 50%.

4. Photosynthetic mutants have a pronounced tendency to revert or become suppressed. They should be cloned and tested on minimal medium frequently. If necessary they can usually be recovered after a backcross to wild type.

6-13-88 Solns

A. Beijerinchs

8 g NH_4Cl
1 ml $CaCl_2$ (1g/ml stock)
1 g $MgSO_4 \cdot 7H_2O$
to 1 l

B Phosphate Buf.

9.25 g KH_2PO_4 Aldrich 99.999%
22,980-6
to 500 ml H_2O
= 0.136 M stock

8-1 $^-SO_4$, ^-Cu Beijerinchs

Gold Label

4 g NH_4Cl
0.5 ml $CaCl_2$
0.82 $MgCl_2 \cdot 6H_2O$ ($= 8 \times 10^{-3}$ m)
to 500 ml w/ H_2O