Chlamydomonas Media. All of these recipes are from the Chlamy.org site, maintained by the University of Minnesota, reproduced here for convenience.

Be sure to check the www.chlamy.org site for updates.

Sager and Granick medium

Originally published by Sager and Granick (1953). Nutritional studies with *Chlamydomonas reinhardi.* *Ann. New York Acad. Sci.* **56**, 831-838
This recipe is a slight modification of their "medium I", as made in the laboratory of Dr. Joel Rosenbaum at Yale. Make the following separate stock solutions:

1. trace elements

H_3BO_3	1.0 g
$ZnSO_4 . 7H_2O$	1.0 g
$MnSO_4 . 4H_2O$	0.30 g
$CoCl_2 . 6H_2O$	0.20 g
$Na_2MoO_4 . 2H_2O$	0.20 g
$CuSO_4$	0.04 g
water to 1 liter	

2. sodium citrate

Na citrate . $2H_2O$	500 g
water to 1 liter	

3. iron chloride

$FeCl_3 . 6H_2O$	10 g
water to 1 liter	

4. calcium chloride

$CaCl_2 . 2H_2O$	53 g
water to 1 liter	

5. magnesium sulfate 300 g
$MgSO_4 . 7H_2O$
water to 1 liter

6. ammonium salt

NH_4NO_3	450 g
or	
NH_4Cl	600 g
water to 1 liter	

Sager's original recipe specified ammonium nitrate; ammonium chloride should be substituted for strains lacking nitrate reductase activity

7. potassium phosphate, monobasic

KH_2PO_4 . $7H_2O$ 200 g
water to 1 liter

8. potassium phosphate, dibasic

K_2HPO_4 . $7H_2O$ 200 g
water to 1 liter

To make the final medium, mix 1.0 ml each of solutions 1 through 6, and 0.5 ml each of solutions 7 and 8.

The final pH should be about 6.9.

To make nitrate medium for testing transformants of *nit1*, omit solution 6, and substitute 4.0 ml of a 1 M solution of KNO_3.

To grow non-photosynthetic strains, add 2.5 ml of a solution of 600 g/l sodium acetate hydrate (NaOAc . $3H_2O$), and increase solutions 7 and 8 to 1.0 ml each to increase the buffering capacity.

TAP medium

from Gorman, D.S., and R.P. Levine (1965) *Proc. Natl. Acad. Sci. USA* **54**, 1665-1669.

This is probably the most widely-used medium at present for experimental work.

Make the following stock solutions:

1. TAP salts

NH_4Cl	15.0 g
$MgSO_4 . 7H_2O$	4.0 g
$CaCl_2 . 2H_2O$	2.0 g
water to 1 liter	

2. phosphate solution

K_2HPO_4	28.8 g
KH_2PO_4	14.4 g
water to 100 ml	

3. **Hutner's trace elements** (see next page and/or follow this link)

To make the final medium, mix the following:

2.42 g Tris
25 ml solution #1 (salts)
0.375 ml solution #2 (phosphate)
1.0 ml solution #3 (trace elements)
1.0 ml glacial acetic acid
water to 1 liter

For solid medium, add 15 g agar per liter

Autoclave.

For 'Tris-minimal' medium: omit acetic acid, titrate final solution to pH 7.0 with HCl

Hutner's trace elements

Hutner et al. (1950) *Proc. Am. Philos. Soc.* **94**, 152-170

This mixture is used both in TAP and in the Sueoka high salt medium (Sueoka's is on the next page).

For a detailed analysis of how well this trace elements solution meets the nutritional requirements of *C. reinhardtii*, see Merchant et al. (2006) *Biochim. Biophys. Acta* **1763**, 578-594.

For 1 liter final mix, dissolve each compound in the volume of water indicated.

The EDTA should be dissolved in boiling water, and the $FeSO_4$ should be prepared last to avoid oxidation.

compound	**amount**	**water**
EDTA disodium salt	50 g	250 ml
$ZnSO_4 . 7 H_2O$	22 g	100 ml
H_3BO_3	11.4 g	200 ml
$MnCl_2 . 4 H_2O$	5.06 g	50 ml
$CoCl_2. 6 H_2O$	1.61 g	50 ml
$CuSO_4 . 5 H_2O$	1.57 g	50 ml
$(NH_4)_6Mo_7O_{24}. 4 H_2O$	1.10 g	50 ml
$FeSO_4. 7 H_2O$	4.99 g	50 ml

Mix all solutions except EDTA. Bring to boil, then add EDTA solution. The mixture should turn green. When everything is dissolved, cool to 70 degrees C. Keeping temperature at 70, add 85 ml hot 20% KOH solution (20 grams / 100 ml final volume). Do NOT use NaOH to adjust the pH.

Bring the final solution to 1 liter total volume. It should be clear green initially. Stopper the flask with a cotton plug and let it stand for 1-2 weeks, shaking it once a day. The solution should eventually turn purp and leave a rust-brown precipitate, which can be removed by filtering through two layers of Whatman#1 filter paper, repeating the filtration if necessary until the solution is clear. Store refrigerated or frozen convenient aliquots. Some people shorten the time for formation of the precipiate by bubbling the solutio with filtered air.

If no precipitate forms, the solution is still usable. However, you might want to check the pH in this case and adjust it to around 7.0 using either KOH or HCl as needed.

To prepare sulfur-free trace elements for hydrogen generation, the sulfate salts can be replaced with equimolar chloride salts ($ZnCl_2$ 10.0 g; $CuCl_2 . 2 H_2O$ 1.00 g; $FeCl_2 . 4 H_2O$, 3.60 g). .

Sueoka's high salt medium

from Sueoka, N. (1960) *Proc. Natl. Acad. Sci. USA* **46**, 83-91.

Also known as HS or HSM, this is somewhat more economical than TAP to prepare.

Make the following stock solutions:

1. salts solution (also known as Beijerinck's solution)

NH_4Cl	100.0 g
$MgSO_4 \cdot 7H_2O$	4.0 g
$CaCl_2 \cdot 2H_2O$	2.0 g
water to 1 liter	

2. phosphate solution

K_2HPO_4	288.0 g
KH_2PO_4	144.0 g
water to 1 liter	

3. **Hutner's trace elements** (follow this link)

To make the final medium, mix the following:

5 ml solution #1 (salts)
5 solution #2 (phosphate)
1.0 ml solution #3 (trace elements)
water to 1 liter

Sodium acetate can be added at 1.2 g/liter (anhydrous) or 2.0 g/liter (hydrate).
For solid medium, add 15 g agar per liter.
Yeast extract 4.0 g/liter can also be added to solid medium.

All these ingredients can be added before autoclaving.

We don't recommend adding yeast extract to liquid media, since it encourages overgrowth of contaminating bacteria.