Chemically Defined *C. elegans* Medium Preparation

Adapted from: Lu & Goetsch (1993) Nematologica 39:303-331
Prepared by: David Hoffman (Conley Lab)

Because of the complexity and length of this media preparation, it is broken down into a series of component preparations which may be prepared in advance and stored frozen at -20°C or -80°C for up to six months.

A. Vitamins and Growth Factor Solutions

1. Water Soluble Components

	2 liters of 2X	4 liters of 2X	8 liters of 2X
N-Acetylglucosamine	0.0600 g	0.1200 g	0.2400 g
Cyanocobalamine (vitamin B_{12})	0.0150 g	0.0300 g	0.0600 g
Niacinamide	0.0300 g	0.0600 g	0.1200 g
Pantethine	0.0150 g	0.0300 g	0.0600 g
Calcium pantothenate	0.0300 g	0.0600 g	0.1200 g
Pyridoxamine dihydrochloride	0.0150 g	0.0300 g	0.0600 g
Pyridoxine hydrochloride	0.0300 g	0.0600 g	0.1200 g
Pyridoxal monophosphate	0.0150 g	0.0300 g	0.0600 g
Riboflavin, sodium salt, 5' phosphate, $2H_20$	0.0300 g	0.0600 g	0.1200 g
Thamine hydrochloride	0.0300 g	0.0600 g	0.1200 g

Place dry chemicals into a 50 ml, 100 ml, or 400 ml beaker, depending upon the amount you're making.

Add about 20 ml, 40 ml, or 80 ml of water and stir until completely dissolved.

Transfer quantitatively to a graduated cylinder or volumetric flask and bring to a final volume of 25 ml, 50 ml, or 100 ml with more water.

Divide into 12.5 ml aliquots (one per liter of 2X medium) and store frozen at either -20°C or -80°C for up to six months in 15 ml conical tubes.

2. TEA-Soluble Components

Before beginning to prepare this solution, first prepare a 10% (w/v) solution of TEA (triethanolamine) by adding 9.2 ml TEA (97%) using a 10 ml pipet to 90.8 ml of distilled water and mixing thoroughly.

	2 liters of 2X	4 liters of 2X	8 liters of 2X
Biotin	0.0150 g	0.0300 g	0.0600 g
Niacin	0.0300 g	0.0600 g	0.1200 g
Folic Acid (Pteroylglutamic acid)	0.0300 g	0.0600 g	0.1200 g
Lipoic Acid (DL-Thioctic acid)	0.0150 g	0.0300 g	0.0600 g
p-Aminobenzoic Acid (PABA)	0.0300 g	0.0600 g	0.1200 g

Place dry chemicals into a 50 ml, 100 ml, or 400 ml beaker, depending on the amount you're making.

Add 1.3 ml, 2.6 ml, or 5.2 ml of 10% (w/v) TEA and 20 ml, 40 ml, or 80 ml of distilled water.
Stir and heat gently to not more than 40°C until dissolved.

Transfer quantitatively to a graduated cylinder or volumetric flask and bring to a final volume of 25 ml, 50 ml, or 100 ml with more water.

Divide into 12.5 ml aliquots (one per liter of 2X medium) and store frozen at either -20°C or -80°C for up to six months in 15 ml conical tubes.

B. Salt Solutions

1) Weigh out the following and place into a beaker:

	0.5 liters of 2X	1 liters of 2X	4 liters of 2X	8 liters of 2X
$CaCl_2 \bullet 2H_2O$	0.2205 g	0.4410 g	1.7640 g	3.5280 g
$CuCl_2 \bullet 2H_2O$	0.0065 g	0.0130 g	0.0520 g	0.1040 g
$MnCl_2 \bullet 4H_2O$	0.0222 g	0.0444 g	0.1776 g	0.3552 g
$ZnCl_2$	0.0102 g	0.0204 g	0.0816 g	0.1632 g

Add not more than 10 ml, 20 ml, or 40 ml of water and stir until dissolved.

2) Weigh out the following and place into a beaker:

	0.5 liters of 2X	1 liters of 2X	4 liters of 2X	8 liters of 2X
KH_2PO_4	1.2255 g	2.4510 g	9.8040 g	19.6080 g
K_3Citrate$\bullet H_20$	0.4860 g	0.9720 g	3.8880 g	7.7760 g
$Fe(NH_4)_2(SO_4)_2 \bullet 6H_20$	0.0588 g	0.1176 g	0.4704 g	0.9408 g

Add not more than 10 ml, 20 ml, or 40 ml of water and stir until dissolved.

3) Weigh out the following and place into a beaker:

	0.5 liters of 2X	1 liters of 2X	4 liters of 2X	8 liters of 2X
$Mg(OH)_2$	0.1740 g	0.3480 g	1.3920 g	2.7840 g
Citric acid$\bullet H_20$	0.6303 g	1.2606 g	5.0424 g	10.0848 g

Add not more than 20 ml, 40 ml, or 80 ml of water and stir until dissolved.

Next, add solution 2) to solution 3), using only 1-2 ml of rinse water to effect the transfer.

Add the dissolved chlorides of solution 1) to the combined salts of solutions 2) and 3), using only 1-2 ml of rinse water. Bring to 45ml with water. If a precipitate forms, continue stirring the mixture until it dissolves.

Each liter of 2X medium requires 90 ml of the combined salt solution.

The final salt solution should be greenish-yellow, and should be frozen at -20°C in 45 ml aliquots using 50 ml conical tubes.

C. Amino Acids

1. Essential amino acids.

Weigh out the ten components listed below and place successively into a beaker.

	0.5 liters of 2X	1 liter of 2X	4 liters of 2X	8 liters of 2X
L-Arginine	0.9750 g	1.9500 g	7.8000 g	15.60 g
L-Histidine	0.2830 g	0.5660 g	2.2640 g	4.528 g
L-Lysine•HCl	1.2830 g	2.5660 g	10.264 g	20.528 g
L-Tryptophan	0.1840 g	0.3680 g	1.4720 g	2.944 g
L-Methionine	0.3890 g	0.7780 g	3.1120 g	6.224 g
L-Threonine	0.7170 g	1.4340 g	5.7360 g	11.472 g
L-Leucine	1.4390 g	2.8780 g	11.512 g	23.024 g
L-Isoleucine	0.8610 g	1.7220 g	6.888 g	13.776 g
L-Valine	1.0200 g	2.0400 g	8.160 g	16.32 g
L-Phenylalanine	0.6230 g	1.2460 g	4.984 g	9.968 g

Add about 85 ml, 170 ml, 680 ml, or 1360 ml of water to the beaker, depending on how much medium you're making.

Stir and heat to between 50°C and 60°C for 3-4 hours or until the amino acids have dissolved into solution. Cool to room temperature before adding to the rest of the media components.

Freeze at -20°C in 170 ml aliquots (one per liter of 2X medium) for up to six months.

2. Nonessential amino acids.

Weigh out the ten components listed below and place successively into a beaker.

	0.5 liters of 2X	1 liter of 2X	4 liters of 2X	8 liters of 2X
L-Phenylalanine*	0.1800 g	0.3600 g	1.440 g	2.880 g
L-Tyrosine*	0.2720 g	0.5440 g	2.176 g	4.352 g
L-Alanine	1.3950 g	2.790 g	11.160 g	22.320 g
L-Aspartic Acid	1.6200 g	3.240 g	12.960 g	25.920 g
L-Cysteine•HCL•H_2O	0.0280 g	0.0560 g	0.2240 g	0.4480 g
L-Glutamate(Na)•H_2O	0.5500 g	1.100 g	4.400 g	8.800 g
L-Glutamine	1.4630 g	2.926 g	11.680 g	23.360 g
Glycine	0.7220 g	1.440 g	5.776 g	11.552 g
L-Proline	0.6530 g	1.306 g	5.224 g	10.448 g
L-Serine	0.7880 g	1.576 g	6.304 g	12.608 g

* Tyrosine is difficult to dissolve, thus it is partially replaced with Phenylalanine

Add about 170 ml, 340 ml, 1360 ml, or 2720 ml of water to the beaker, depending on how much medium you're making.

Stir and heat to between 50°C and 60°C for 3-4 hours or until the amino acids have dissolved into solution. Cool to room temperature before adding to the rest of the media components.

Freeze at -20°C in 340 ml aliquots (one per liter of 2X medium) for up to six months.

D. Nucleic Acid Substituents

Weigh out the following components and place successively into a beaker.

	0.5 liters of 2X	1 liter of 2X	4 liters of 2X	8 liters of 2X
Adenosine-3'(2')-phosphoric acid•H_2O	0.3652 g	0.7304 g	2.9216 g	5.8432 g
Cytidine-3'(2')-phosphoric acid	0.3232 g	0.6464 g	2.5856 g	5.1712 g
Guanosine-3'(2')-phosphoric acid-$PO_4(Na)_2$•H_2O	0.3632 g	0.7264 g	2.9056 g	5.8112 g
Uridine-3'(2')-phosphoric acid	0.3242 g	0.6484 g	2.5936 g	5.1872 g
Thymine	0.1261 g	0.2522 g	1.0088 g	2.0176 g

Add about 20 ml, 40 ml, 160 ml, or 320 ml of distilled water while stirring.

Next, add 0.617 ml, 1.234 ml, 4.936 g, or 9.872 ml of 10% (w/v) KOH solution, and then add an additional 65 ml, 130 ml, 520 ml, or 1040 ml of distilled water and heat to ~50°C until the nucleic acid substituents have dissolved. Cool before adding to the rest of the medium components.

Freeze at -20°C in 170 ml aliquots (one per liter of 2X medium) for up to six months.

E. Other Growth Factors

	0.5 liters of 2X	1 liter of 2X	4 liters of 2X	8 liters of 2X
Glutathione (reduced)	0.2040 g	0.4080 g	1.632 g	3.264 g
Choline•H_2Citrate	0.0885 g	0.1770 g	0.7080 g	1.416 g
myo-inositol	0.0645 g	0.1290 g	0.5160 g	1.032 g
Cytochrome C (Type III)	0.0500 g	0.100 g	0.400 g	0.800 g

1. β-sitosterol

β-sitosterol, the only lipid component of the medium, does not dissolve in an aqueous solution, so a nonionic detergent (Tween 80) is used as the solvent. Prepare a 50 ml β-sitosterol stock solution as follows:

Prepare 9 tubes with screw caps or tightly fitting culture caps for later use.

Weigh out 0.2500 g of β-sitosterol in a small beaker.

Add precisely 6.25 ml of Tween 80 using a 10 ml TD pipette.

Heat and stir the mixture on a stir plate until the solute is completely dissolved (~10 minutes).

Remove from heat and slowly add about 30 ml of distilled water to he mixture while stirring,
and bring the solution up to a final volume of 50 ml using a graduated cylinder or volumetric flask.

Pipet 5.5 ml β-sitosterol solution into each of 9 glass test tubes and cap them loosely.

Autoclave the tubes at 122°C/250°F and 15 p.s.i. for 15 minutes on slow exhaust.

After autoclaving, the solution will display two separate layers of different density**.**
Immediately vortex the tubes thoroughly when still very hot until the solution is completely clear.
This stabilizes the emulsion.

Cool the tubes to room temperature. Four tubes of this stock solution are needed for each liter of 2X medium.

Prepare this solution each time the medium is compounded.

F. Energy Source

	0.5 liter of 2X	1 liter of 2X	4 liters of 2X	8 liters of 2X
D-glucose	32.50 g	65.00 g	260 g	520 g
KCH_3COOH	5.0 g	10.0 g	40.0 g	80.0 g

Note: Use either D-glucose or KCH_3COOH; it's not necessary to use both.

G. Compounding the medium.

Set up a beaker of the appropriate size on a magnetic stir plate.

Transfer the essential amino acid solution, the nonessential amino acid solution, and the nucleic acid substituent solution into the beaker using no more than a few ml of rinse water to effect the transfer and continue stirring vigorously as the remaining components are added.

Transfer the combined salt solutions into the beaker, using no more than a couple ml of rinse water to effect the transfer.

Pipet 12.5 ml of the water-soluble vitamins and growth factors solution into the beaker, followed by 12.5 ml of the TEA-soluble vitamins and growth factors solution for each liter of 2X medium.

Next, add successively the weighed dry components (reduced glutathione, choline, *myo*-inositol, Cytochrome C, and D-glucose or KCH_3COOH).

Finally, add the β-sitosterol stock solution (20 ml for each liter of double-strength medium).

Warm the medium to ~30°C with stirring for about an hour to facilitate the dissolution of all components. The pH of the combined solutions is about 4.3. Add 10% (w/v) KOH until the desired pH=5.9±0.1 is reached.

Using either a graduated cylinder or a volumetric flask, bring the medium up to the appropriate final volume with distilled water, mix thoroughly, and filter sterilize using a Millipore 0.2 μm cellulose acetate filter. If you wish to make 1X medium, dilute the solution 1:1 with distilled water and mix thoroughly before sterile-filtering.

Store the sterile medium in appropriate light tight (or foil-wrapped) containers at -20°C for up to six months.