

Procedure and notes

Note, there are 4 chemicals that do not require a stock, and instead the solid is added directly to the beaker.

The solutions highlighted in blue MUST be made on the same day that the buffered base is made.

Fill a clean beaker with volume of dH₂O stated below, and add the appropriate volume of stock, or add solid directly to beaker.

pH to 6.8 with HCl, add dH₂O up to required volume, and filter sterilize. Wrap in foil, store at 4C for up to 2 weeks.

On day of use add supplements (sugars, iron, CaCl_2 , MgCl_2 , vitamins).

For mucin+ add mucin dissolved in H₂O to buffered base before filter sterilisation.

Green cells alter to desired volume

1000 final volume (ml)

chemical

chemical unit	name	stock M	stock volume ml	MW g/mol	mass to add g	stock to add to beaker ml	final mM	notes
NaH2PO4	sodium phosphate monobasic	0.2	6.500	119.98	0.156	6.500	1.3	add solid directly
Na2HPO4	sodium phosphate dibasic	0.2	6.250	141.98	0.177	6.250	1.25	add solid directly
KNO3	Potassium nitrate	1	0.348	101.11	0.035	0.348	0.348	add solid directly
K2SO4	Potassium sulfate	0.25	1.084	174.26	0.047	1.084	0.271	add solid directly
NH4Cl	Ammonium chloride			53.49	0.122		2.2808	add solid directly
KCl	Potassium chloride			74.55	1.114		14.943	add solid directly
NaCl	Sodium chloride			58.44	3.030		51.848	add solid directly
MOPS	MOPS buffer			209.26	2.093		10	add solid directly

chem

Chemical unit	name	stock M	stock volume ml	new g/mol	mass to add g	stock to add to beaker ml	ml mM	notes
Ser	Serine	0.1	14.460	105.09	0.152	14.460	1.446	add solid directly
Glu * HCl	Glutamic acid hydrochloride	0.1	15.490	183.59	0.284	15.490	1.549	add solid directly
Pro	Proline	0.1	16.610	115.13	0.191	16.610	1.661	add solid directly
Gly	Glycine	0.1	12.030	75.07	0.090	12.030	1.203	add solid directly
Ala	Alanine	0.1	17.800	89.09	0.159	17.800	1.78	add solid directly
Val	Valine	0.1	11.170	117.15	0.131	11.170	1.117	add solid directly
Met	Methionine	0.1	6.330	149.21	0.094	6.330	0.633	add solid directly
Ile	Isoleucine	0.1	11.210	131.17	0.147	11.210	1.121	add solid directly
Leu	Leucine	0.1	16.090	131.17	0.211	16.090	1.609	add solid directly
Orn * HCl	Ornithine hydrochloride	0.1	6.760	168.62	0.114	6.760	0.676	add solid directly
Lys * HCl	Lysine hydrochloride	0.1	21.280	182.65	0.389	21.280	2.128	add solid directly
Arg * HCl	Arginine hydrochloride	0.1	3.060	210.66	0.064	3.060	0.306	add solid directly
Asp	Aspartic acid	0.1	20.000	133.1	0.266	8.270	0.827	Prep in 0.5 M NaOH

Prepare these stocks fresh

Trp	Tryptophan	0.1	10	204.23	0.204	0.130	0.013	Prep in 0.2 M NaOH
Tyr	Tyrosine	0.1	20	181.19	0.362	0.020	0.802	Prep in 1.0 M NaOH
Thr	Threonine	0.1	10.720	119.12	0.128	10.720	1.072	add solid directly
Cys * HCl	Cysteine hydrochloride	0.1	1.600	157.62	0.025	1.600	0.16	add solid directly
Phe	Phenylalanine	0.1	5.300	165.19	0.088	5.300	0.53	add solid directly
His * HCl	Histidine hydrochloride	0.1	5.190	209.63	0.109	5.190	0.519	add solid directly

500 Add above to this vol dH₂O (ml), pH to 6.8 with HCl and **top up to required volume**, filter, wrap in foil and store at 4C for up to 2 weeks

Make up stocks listed below. The FeSO_4 stock must be made fresh; do not use if precipitation or color change is observed.

100 Add these to this volume of SCFM (ml) base on the same day as media is to used.

chemical unit	name	stock M	stock volume ml	MW g/mol	mass to add g	stock to add to SCFM base ul	final mM	notes
G-glucose	Glucose	1	5	180.16	0.90	300.0	3	sterile filter
L-lactic acid	Lactic acid	1	5	90.08	0.45	930.0	9.3	sterile filter, pH stock to 7 with NaOH
CaCl2 * H2O	Calcium chloride	1	5	147.01	0.74	175.4	1.754	sterile filter
MgCl2	Magnesium chloride	1	5	95.21	0.48	60.6	0.606	sterile filter
FeSO4·7H2O	Iron (II) sulfate heptahydrate	0.0036	15	278.01	0.02	100.0	0.0036	sterile filter and make fresh

Vitamins: Make up stocks listed below, store in the dark at -20C in 1 ml aliquots, thaw once only

100 Add these to this volume of SCFM (ml) base on the same day as media is to be used

chemical unit	name	stock mg/L	stock volume ml	mass to add g	stock to add to SCFM base ul	final mg/L	notes
Thiamine * HCl	Thiamine hydrochloride (B1)	1000	30	0.03	100	1.000	sterile filter
Niacin	Niacin acid (B3)	1000	30	0.03	120	1.200	sterile filter
Calcium pantothenate	D-Pantothenic acid hemicalcium salt (B5)	1000	30	0.03	25	0.250	sterile filter
Biotin	Biotin (B9)	500	40	0.02	1	0.005	sterile filter, add small amounts of 1N NaOH until biotin dissolves

NaOH for amino acids and Biotin

chemical unit	name	stock M	stock volume ml	MW g/mol	mass to add g
NaOH	Sodium hydroxide	0.2	10	40	0.08
NaOH	Sodium hydroxide	0.5	20	40	0.40
NaOH	Sodium hydroxide	1	20	40	0.80

Other SCFM based media:

For SCFM + mucin

Dissolve this weight of mucin in this volume of sterile dH₂O, add mucin slowly to the water, can be left at 4°C overnight to dissolve

Chemical	name	weight g	volume ml
Mucin	Mucin porcine stomach (type II)	5	250

Final concentration of mucin: 5g/L

Add mucin dissolved in H₂O to buffered base before filter sterilisation - filter sterilise as normal, will take longer than normal

For SCFM agar plates

Autoclave this weight of agar in this volume of H₂O

chemical	name	weight g	volume ml
Agar	Agar	15	500

Rather than dissolving the buffered base in stated volume of water dissolve in this volume instead:

300 ml

Add amino acids than make up to half the desired volume before filtering as normal:

500 ml

Sterilely mix the autoclave agar (once cooled to below 60C) with the filtered SCFM, quickly add premixed supplements and pour plates