YEAST AND BACTERIAL MEDIA RECIPES

10X YEAST NITROGEN BASE SOLUTION (YNB + dextrose + $(NH_4)_2SO_4$)

Dissolve 1.7 g yeast nitrogen base (w/o ammonium sulfate and w/o amino acids) and 5 g of $(NH_4)_2SO_4$ in 100 ml of distilled water, filter sterilize and transfer sterilely to a sterile bottle or to autoclaved medium. The same sterile filter unit can be used for the to sterilize additional YNB etc. unless the spout is contaminated by coming into contact with a nonsterile surface.

10X SUPPLEMENT SOLUTIONS

Dry supplement mix is dissolved in 100 ml distilled water (when making 1000 ml of medium). This should be done on a stir plate set at low heat. Once the powder has dissolved (a few crystals may be left after 30 min. of stirring) the mixture is filter sterilized and can then be added to the appropriate autoclaved media. The following amounts of supplement mix are used to make one liter of media:

SUPPLEMENT MIX	WEIGHT FOR 1 LITER OF MEDIA	TYPE OF MEDIA
-ADE	440 mg	SDC-ADE
-ARG	440 mg	SDC-ARG, SDC-ARG+CAN
-HIS	440 mg	SDC-HIS, SORB/SDC-HIS
-LEU	430 mg	SDC-LEU, SORB/SDC-LEU
-LYS	430 mg	SDC-LYS
-MET	440 mg	SDC-MET
-THR	260 mg	SDC-THR
-TRP	440 mg	SDC-TRP, SORB/SDC-TRP
-TYR-PHE	380 mg	SDC-TYR-PHE
-URA	440 mg	SDC-URA, SORB/SDC-URA
COM	460 mg	SDC, Kac

AMINO ACID AND BASE STOCK SOLUTIONS

Stock solutions are given as weight/volume (g/100 ml) and are made in H_2O unless otherwise noted. All solutions should be stored in the refrigerator. Arginine, aspartic acid, histidine, threonine, tryptophan, tyrosine, canavanine and cycloheximide solutions should be filter sterilized rather than autoclaved. Histidine and tryptophan solutions should be kept in the dark.

name/concentration/made in	ml of solution/l of medium & ~ µl/plate (assume 30 plates/l)
0.5% adenine/0.05 N HCl	8 ml/l & 280 µl/plate (neutralize with 0.4 ml of 1 M base/l after adding to medium)
2% arginine	1 ml/l & 35 µ/l/plate
2% aspartic acid/adjust to pH 5.5	5 ml/l & 175 µl/plate
2% histidine	1 ml/l & 35 µl/plate
0.6% isoleucine	5 ml/l & 175 µl/plate
1% leucine	6 ml/l& 210 µl/plate
1.5% lysine	2 ml/l & 70 µl/plate
2% methionine	1 ml/l & 35 µl/plate
2% phenylalanine	2 ml/l & 70 µl/plate
6% threonine	5 ml/l & 175 µl/plate
1% tryptophan	2 ml/l & 70 µl/plate
0.25% tyrosine/0.05 N NaOH	10 ml/l & 350 µl/plate (neutralize with 0.5 ml of 1 M HCl/l after adding to medium)
0.2% uracil/1% Na_2CO_3	5 ml/l & 175 µl/plate
3% valine	5 ml/l & 175 µl/plate

MISCELLANEOUS STOCK SOLUTIONS

2% canavanine	2 ml/l & 70 µl/plate
1% cycloheximide	1 ml/l & 35 µl/plate
1M (= 8.4%) 3-aminotriazole /95% EtOH	add to media in the 10 - 50 mM range put on roller drum @ 37˚C to get into solution
0.5% trifluoroleucine	
1% mimosine (0.05 N NaOH)	
10% hygromycin B	
10% G418	
1% S-2-aminoethyl-L-cysteine (thiosine)	add to final concentration of 100 µg/ml
0.5% β-thienylalanine	
1% β-chloroalanine	
0.4% ONPG	

1000X VITAMIN STOCK SOLUTION

Vitamin	Stock solution	Vol. added for 100 ml of 1000X vitamin stock solution; stocks may have to warmed to go into solution; solubility ()
biotin	0.01 g/100 ml 95% EtOH	2 ml (~ 80 mg/100 ml 95% EtOH)
calcium pantothenate	1 g/100 ml	4 ml (1 g/2.8 ml H_2O)
folic acid	0.01 g/100 ml	2 ml (warm to dissolve)
inositol	10%	2 ml (14 g/100 ml H_2O)
niacin	1%	4 ml (1 g/60 ml EtOH or 1.4 ml H_2O)
p-aminobenzoic acid	1 g/100 ml 95% EtOH	2 ml
pyridoxine HCl	1 g/100 ml 95% EtOH	4 ml (1 g/90 ml EtOH or 4.5 ml H_2O)
thiamine HCl	1 g/100 ml 95% EtOH	4 ml (1 g/100 ml 95% EtOH or 1 ml H_2O)
riboflavin		add 20 mg

Complete vitamin solution:

1.) Remove the individual stock solutions from the refrigerator, may have to warm to get them back into solution,
2.) Combine all 95% EtOH vitamin solutions (12 ml volume),
3.) Add 20 mg riboflavin to 95% EtOH mixture,
4.) Combine all filter sterilized H_2O solutions (12 ml volume) with 76 ml sterile dH_2O,
5.) Combine the sterile H_2O solution with the 95% EtOH solution/suspension for a final volume of 100 ml. Add 1 ml to 1 l of medium.

Vitamin dropout solutions:

Same as above except that one or more vitamins are not added to the final mixture and the volume of sterile dH_2O added is adjusted so the final volume is 100 ml. Add 1 ml to 1 l of medium.

VITAMIN CONCENTRATIONS IN DEFINED MEDIUM

Vitamin	Final conc.
biotin	2 µg/l
calcium pantothenate	400 µg/l
folic acid	2 µg/l
inositol	2 mg/l
niacin	400 µg/l
p-aminobenzoic acid	200 µg/l
pyridoxin HCl	400 µg/l

riboflavin	200 µg/l
thiamine HCl	400 µg/l

MAKING SUPPLEMENT MIXTURES

There are ten different dropout mixtures. Nine of these lack a single amino acid or base (ADE, ARG, HIS, LEU, LYS, MET, THR, TRP and URA) and one is missing two amino acids (TYR-PHE). There is also a complete mixture (COM) which contains everything. To make a supplement mixture simply weigh out the different components (minus 1 or 2) and grind them into a fine powder with a mortar and pestle (~ 4-5 minutes) or a coffee grinder (clean thoroughly after each use). One mixture made according to this recipe will make ~ 40 liters of plates. Charts have been made for each kind of supplement mix. You should make copies of all of the charts. When you are making up a supplement mix check off each component on the chart. This makes it less likely that a component will be left out or added twice. Another useful trick: after weighing out each component (on a separate weigh boat or piece of weigh paper), place it on the bench and label each one e.g. adenine, arginine, etc. When you are done recheck to make sure you have all the components, then combine them and mix them. Store the supplement mixtures in disposable plastic scintillation vials; mark the amount to be used per liter of media on each vial.

SUPPLEMENT MIXTURE COMPONENTS

adenine	800 mg
arginine	800 mg
histidine	800 mg
leucine	1200 mg
lysine	1200 mg
methionine	800 mg
phenylalanine	2000 mg
threonine	8000 mg
tryptophan	800 mg
tyrosine	1200 mg
uracil	800 mg

COMPONENT	TYPE OF SUPPLEMENT MIX			
	-ADE	-ARG	-HIS	-LEU
adenine	0 mg	800 mg	800 mg	800 mg
arginine	800 mg	0 mg	800 mg	800 mg
histidine	800 mg	800 mg	0 mg	800 mg
leucine	1200 mg	1200 mg	1200 mg	0 mg
lysine	1200 mg	1200 mg	1200 mg	1200 mg
methionine	800 mg	800 mg	800 mg	800 mg
phenylalanine	2000 mg	2000 mg	2000 mg	2000 mg
threonine	8000 mg	8000 mg	8000 mg	8000 mg
tryptophan	800 mg	800 mg	800 mg	800 mg
tyrosine	1200 mg	1200 mg	1200 mg	1200 mg
uracil	800 mg	800 mg	800 mg	800 mg

COMPONENT	TYPE OF SUPPLEMENT MIX			
	-LYS	-MET	-THR	-TRP
adenine	800 mg	800 mg	800 mg	800 mg
arginine	800 mg	800 mg	800 mg	800 mg
histidine	800 mg	800 mg	800 mg	800 mg
leucine	1200 mg	1200 mg	1200 mg	1200 mg
lysine	0	1200 mg	1200 mg	1200 mg
methionine	800 mg	0	800 mg	800 mg
phenylalanine	2000 mg	2000 mg	2000 mg	2000 mg
threonine	8000 mg	8000 mg	0	8000 mg
tryptophan	800 mg	800 mg	800 mg	0
tyrosine	1200 mg	1200 mg	1200 mg	1200 mg
uracil	800 mg	800 mg	800 mg	800 mg

COMPONENT	TYPE OF SUPPLEMENT MIX		
	-TYR-PHE	-URA	COM
adenine	800 mg	800 mg	800 mg
arginine	800 mg	800 mg	800 mg
histidine	800 mg	800 mg	800 mg
leucine	1200 mg	1200 mg	1200 mg
lysine	1200 mg	1200 mg	1200 mg
methionine	800 mg	800 mg	800 mg
phenylalanine	0	2000 mg	2000 mg
threonine	8000 mg	8000 mg	8000 mg
tryptophan	800 mg	800 mg	800 mg
tyrosine	0	1200 mg	1200 mg
uracil	800 mg	0	800 mg

PLATE RECIPES

SD PLATES:

Agar	YNB + Dextrose
850 ml distilled water	150 ml distilled water
20 g. agar:	6.7 g Yeast Nitrogen Base (w/o amino acids, with ammonium sulfate)
Autoclave	20 g. Dextrose
	Filter Sterilize

After autoclaving combine the autoclaved solution and 150 ml of (filter sterilized) Yeast Nitrogen Base etc. Mix thoroughly and pour plates.

SDC and SDC-? PLATES:

Agar	YNB + Dextrose + Amino Acids
850 ml distilled water	150 ml distilled water
20 g. agar	Amino acid supplement mixture
Autoclave	6.7 g Yeast Nitrogen Base (w/o amino acids, with ammonium sulfate)
	20 g. Dextrose
	Filter Sterilize

After autoclaving combine the autoclaved solution YNB + Dextrose + Amino Acids. Mix thoroughly and pour plates.

SDC - ARG + CAN PLATES:

1.) make SDC-ARG (see SDC-? above)

2.) after autoclaving add 2 ml of 2% canavanine (filter sterilized), do not mouth pipette

2% canavanine solution: Wear gloves when making the solution. Dissolve 2 g canavanine in 100 ml of distilled water, filter sterilize and transfer 10 to 15 ml to sterile plastic tubes (15 ml). Keep one tube in the refrigerator ready for use. The other tubes should be kept frozen. When a frozen tube is thawed it must be thoroughly mixed, otherwise the canavanine will be concentrated in the bottom of the tube.

SORBITOL CONTAINING DROPOUT PLATES: SORB/SDC-?

Agar	YNB + Dextrose + Amino Acids
670 ml distilled water:	150 ml distilled water
182 g Sorbitol	Amino acid supplement mixture
20 g. agar	6.7 g Yeast Nitrogen Base (w/o amino acids, with ammonium sulfate)
Autoclave	20 g. Dextrose
	Filter Sterilize

After autoclaving combine the autoclaved solution YNB + Dextrose + Amino Acids. Mix thoroughly and pour plates.

YEPD (YPD) PLATES:

Agar
20 g. Peptone
10 g. Yeast Extract
900 ml distilled water:
5 ml of 1 M HCl (do not mouth pipette)
20 g. Agar
Autoclave

20% Dextrose
100 ml distilled water
20 g. Dextrose
Autoclave

After autoclaving mix the two components and pour plates

Making 1 M HCl: This entire operation must be done in a hood and gloves and lab coat must be worn. Do not even think about mouth pipeting concentrated hydrochloric acid. Measure 83 ml of concentrated hydrochloric acid (HCl) in a graduated cylinder and slowly add the HCl to 917 ml of distilled water which is stirring on a stir plate. Once the 1 M HCl is made up it can be transferred to a plastic 1 liter bottle. The graduated cylinder which is used to measure the concentrated HCl must be carefully rinsed out with water before being put in with dirty glassware to be cleaned.

YEPD+CYH PLATES:
1.) make YEPD (as above)
2.) add 1 ml of 1% cycloheximide (filter sterilized) after autoclaving, do not mouth pipet

1% cycloheximide solution: Wear gloves when making up the solution. Dissolve 1 g cycloheximide in 100 ml of distilled water, filter sterilize and transfer 10 to 15 ml to sterile plastic tubes (15 ml). One tube is kept refrigerated ready for use while the other tubes are kept frozen. When a frozen tube is thawed it must be thoroughly mixed, otherwise the cycloheximide will be concentrated in the bottom of the tube.

KAc PLATES

Agar
900 ml distilled water
2.2 g yeast extract
0.5 g dextrose
20 g potassium acetate
20 g agar
Autoclave

Amino Acid Supplements
100 ml distilled water
460 mg COM(complete) mixture
Filter sterilize

Combine the autoclaved and filter sterilized solutions, mix thoroughly and pour the plates

DIET KAc PLATES:
1000 ml distilled water
20 g potassium acetate
20 g agar
Autoclave

After autoclaving mix thoroughly and pour plates

YEP(GAL), (MAL), (RAF) AND (SUC) PLATES:

Agar
20 g. Peptone
10 g. Yeast Extract
900 ml distilled water:
5 ml of 1 M HCl (do not mouth pipet)
20 g. Agar
Autoclave

20% Sugar
100 ml distilled water
20 g. Sugar
Filter sterilize

20% sugar solutions consist of 20 g of the appropriate sugar dissolved in 100 ml of distilled water (low heat will help galactose and raffinose go into solution). The solutions are then filter sterilized. After autoclaving add 100 ml of (filter sterilized) 20% galactose or maltose or raffinose or sucrose (substituting raffinose for sucrose allows better scoring of SUC markers); add antimycin A to a final concentration of 0.1µg/ml

YEP(EG) PLATES:

In a 1 L beaker
950 ml distilled water
10 g succinic acid (dissolve succinic first)
20 g bacto-peptone (Sigma S-7501)
20 g glycerol
10 g yeast extract
adjust to pH 5.5 by adding KOH pellets

Transfer pH's media into Flask/Bottle
20 g agar
Autoclave

After autoclaving add 25 ml 95% EtOH, mix thoroughly and pour plates

GELATIN MEDIUM:

In a >2 L container
950 ml distilled water
10 g yeast extract
20 g dextrose

100 g gelatin (Sigma, G-2500; 300 Bloom))

Melt gelatin in 55˚C water bath, autoclave. After autoclaving, mix thoroughly and pour plates

α-AMINOADIPATE MEDIUM (α-AA) PLATES:

Agar
800 ml distilled water:
20 g. Agar
Autoclave

YNB + Dex + Lys
100 ml distilled water
1.7 g. Yeast Nitrogen Base, without $(NH_4)_2SO_4$
20 g dextrose
2 ml of 1.5% lysine
Filter Sterilize

α-aminoadipate
100 ml distilled water
2 g α-aminoadipate
Adjust to pH 5.5. (This will require adding pellets of KOH until all of the powder goes into solution and then adjusting the pH down to 5.5)
Filter sterilize.

After autoclaving, add filter sterilized solutions to autoclaved agar, mix thoroughly and pour plates.

It is possible to add some nutritional supplements to α-AA medium but the best results are obtained with either no supplements (using a prototrophic strain) or supplements that either cannot be utilized as nitrogen sources or are poorly utilized (e.g. adenine, histidine, tryptophan, uracil).

5-FOA PLATES

Agar
500 ml distilled water:
20 g. Agar
Autoclave

YNB + Dex + 5-FOA
500 ml distilled water
6.7 g. Yeast Nitrogen Base, with $(NH_4)_2SO_4$
1 g. 5-fluoroortic acid
10 ml of 0.2% uracil (50 mg)
20 g dextrose
Adjust to pH 4.5
Filter Sterilize

After autoclaving, add filter sterilized solution to autoclaved agar, mix thoroughly and pour plates.

It is possible to add other nutritional supplements or to add dropout mixtures to 5FOA medium. These should be dissolved along with the yeast nitrogen base etc. and filter sterilized.

Personal communication, W.M. Barnes:
- "I have discovered that FOA selection breaks down above pH 4.5. This problem is consistent with only the protonated form of FOA permeating cells. It turns out that the standard recipe for

FOA plates is pH 2.8. Some of this acidity is due to the FOA, which you have less of, so your plates must be at slightly higher pH.
- 5-FOA has no effect at all at pH 6 or 6.2, pretty good at pH 5.4, and full effect at 4 and below. This is as 2X filtered medium at R.T., before mixing with 4% hot agar. After solidifying, the plates have a slightly higher pH, as measured with a pH stick/paper."

D-HISITIDINE PLATES:

Agar	D-Histidine	YNB + Dex + Pro
800 ml distilled water:	100 ml distilled water	100 ml distilled water
20 g. Agar	2.1 g D-histidine	1.7 g. Yeast Nitrogen Base, without $(NH_4)_2SO_4$
Autoclave	Filter sterilize	1 g. Proline
		20 g dextrose
		Filter Sterilize

After autoclaving, add filter sterilized solutions to autoclaved agar, mix thoroughly and pour plates. Final concentration of D-histidine ~ 10 mM.

This medium is used to positively select gap1 mutants. Gap1 is the only amino acid permease which can take up (i) toxic D-amino acids and (ii) citrulline. Therefore, gap1 mutants are (i) resistant to D-amino acids and (ii) unable to utilize citrulline as either a nitrogen source or as an arginine precursor. The general amino acid permease (Gap1) is repressed on rich nitrogen sources hence the use of proline as a nitrogen source.

MMS PLATES:

Make YEPD or SD/SDC medium, allow the medium to cool.

Add methyl methanesulfonate (MMS; methanesulfonic acid methylester, Sigma) to a final concentration of 0.035%. Use the plates ~ 12 hours after pouring them; discard unused plates after 2 days because the MMS breaks down. The best strategy for the occasional use of MMS plates is to have aliquots (e.g. 100 ml) of sterile medium (e.g. YEPD) made up. This sterile medium can be melted, allowed to cool and then MMS can be added. Original recipe is from Genetics 86: 33-55 (1977).

CASEIN PLATES:

Agar	Casein	YNB + Dex
500 ml distilled water:	400 ml distilled water	100 ml distilled water
20 g. Agar	5 g. casein (Sigma C-0376)	1.7 g. Yeast Nitrogen Base, without $(NH_4)_2SO_4$
Autoclave	~ 800 mg (8 pellets) of NaOH is added to help dissolve the casein.	20 g Dextrose
		Filter Sterilize
	Adjust to pH 10 with 1 M HCl	
	Autoclave	

After autoclaving, mix the sterile solutions and pour plates.

DOMINANT DRUG SELECTION MEDIA

YPD + DRUG PLATES:

Use recipe for YPD plates, after autoclaving, cool media to pouring temperature and add 1 ml 1000X stock per liter of media* and pour plates.

SEC or SEC-? + DRUG PLATES:

Agar	YNB + Dextrose + Amino Acids
850 ml distilled water:	150 ml distilled water
20 g. agar	Amino acid supplement mixture
Autoclave	1.7 g. Yeast Nitrogen Base, without $(NH_4)_2SO_4$
	1 g. Glutamic Acid (monosodium salt, Sigma G-1626)
	20 g. Dextrose
	Filter Sterilize

After autoclaving combine the autoclaved solution and 150 ml of (filter sterilized) yeast nitrogen base etc. Cool to pouring temperature, add filter sterilized antibiotic, mix thoroughly and pour plates.

Antibiotic in plates	1000X Stock concentration	Final concentration
Nourseothricin (Nat)	100 mg/ml in ddH_20	100 µg/ml
Geneticin (G418)	200 mg/ml in ddH_20	200 µg/ml
Hygromycin B (Hyg)*	Check the container to determine the concentration	300 µg/ml

Notes:

Often the Hygromycin B is >300 mg/ml, you may have to calculate the amount required for a 300 µg/ml final concentration.

There can be variations on how much drug is added depending upon the strain of yeast or type of media.

SDP PLATES + Bialaphos or Glufosinate:

Agar	YNB + Dextrose
850 ml distilled water	150 ml distilled water
20 g. agar	1.7 g Yeast Nitrogen Base (w/o amino acids, w/o ammonium sulfate)
Autoclave	1 g/L L-Proline
	20 g. Dextrose
	Filter Sterilize

After autoclaving combine the autoclaved solution and 150 ml of (filter sterilized) yeast nitrogen base etc. Cool to pouring temperature, add filter sterilized antibiotic, mix thoroughly and pour plates.

Antibiotic	Final concentration
Bialaphos	200 µg/ml
Glufosinate	600-800 µg

SLAD PLATES:

Agar	YNB + Dex + $(NH_4)_2SO_4$
900 ml distilled water:	100 ml distilled water
20 g. Agar	1.7 g. Yeast Nitrogen Base, without $(NH_4)_2SO_4$
Autoclave	5 ml 10 mM $(NH_4)_2SO_4$
	20 g dextrose
	Filter Sterilize

10 mM $(NH_4)_2SO_4$ = 0.132 g per 100 ml. Alternatively, add 6.6 mg of $(NH_4)_2SO_4$ to 1 l of medium.

After autoclaving, mix the sterile solutions and pour into petri dishes.

Pour plates thin!! Cells on a thin plate starve faster and it's easier to see (& photograph) the pseudohyphae.

Bacterial Media

LB Media (1L)

LB Broth
10 g Bacto-Tryptone
5 g Bacto-Yeast Extract
5 g NaCl
ddH_20 to 1 Liter
Autoclave

LB Plates
10 g Bacto-Tryptone
5 g Bacto-Yeast Extract
5 g NaCl
15 g Agar
ddH_20 to 1 Liter
Autoclave

LB + Antibiotic Plates
- Use recipe for LB plates, after autoclaving, cool media to pouring temperature and add 1 ml 1000X antibiotic stock per liter of media.

Antibiotic	1000X Stock concentration	Final concentration in plates
Ampicillin	100 mg/ml in ddH_20	100 µg/ml
Carbenicillin	100 mg/ml in ddH_20	100 µg/ml
Chloramphenicol	34 mg/ml in 100% EtOH	34 µg/ml
Kanamycin	40 mg/ml in ddH_20	40 µg/ml
Tetracycline	15 mg/ml in 70% EtOH	15 µg/ml
Streptomycin	50 mg/ml in ddH_20	50 µg/ml
Spectinomycin	50 mg/ml in ddH_20	50 µg/ml

Notes:

There can be variations on how much antibiotic is added (and the differences in the concentration of the stocks). I put down my "standard" concentrations. YMMV

LB + X-gal + Antibiotics
- Use recipe for LB plates, after autoclaving, cool media to pouring temperature, add desired antibiotics and add 2 ml of X-gal 20% stock (20 mg/ml) per liter of media. Warning: X-gal is in DMF (Dimethyl Formamide). Do not get DMF on skin, use gloves and eye protection.