Media Recipes

NOTE 1: Recipes are for liquid media (and per L unless stated otherwise). To sterilizate of 1L, autoclave 20-30 minutes (121ºC)

NOTE 2: For solid medium (used for plates) add 20g Agar / L. After autoclaving cool the agar to ≈ 50°C (just about cool enough to hold in your palm) and add other ingredients (such as antibiotics) as needed. At this temperature, the medium will stay liquid indefinitely, but will rapidly solidify if its temperature falls much below 45°C. Ensure agar is mixed well before pouring (spray with 70% EtOH and flame to remove the bubbles: **Careful of flamethrower**). Pour into sterile disposable petri plates (≈ 25mls per) and allow to solidify.

Freshly poured plates are wet and unable to absorb liquid spread onto them. Moreover, plates that are even slightly wet tend to exude moisture and give really crappy colonies. So for most applications, dry the plates by leaving at RTº for 2 or 3 days, or leaving them with the lids off for an hour or two in a laminar flow hood. Store dry plates inverted in stacks at 4°C, wrapped in the original bags used to package the empty plates.

In order:

Dropout Add-Backs

30mM ADE 0.55 g Adenine-hemisulfate, ddH_2O to 500 ml and autoclave
(use at 10 ml ADE stock per L / spread 300µl per add-back plate)

100mM HIS 10.45g L-Histindine-HCL, ddH_2O to 500 ml and autoclave
(use at 3ml HIS stock per L / spread 120µl per add-back plate)

100mM LEU 6.55 g L-Leucine, ddH_2O to 500 ml and autoclave
(use at 16.7 ml LEU stock per L / spread 500µl per add-back plate)

100mM LYS 9.15 g L-Lysine, ddH_2O to 500 ml and autoclave
(use at 10 ml LYS stock per L / spread 300µl per add-back plate)

40mM TRP 4.0 g L-Tryptophan, ddH_2O to 500 ml and autoclave
NB. Foil wrap to protect from light
(use at 10 ml TRP stock per L / spread 300µl per add-back plate)

20mM URA 1.12 g Uracil, ddH_2O to 500 ml and autoclave
(use at 10 ml URA stock per L / spread 300µl per add-back plate)

Other Additives

10% Casamino acids 10g Casamino acids, ddH_2O to 100 ml and autoclave

200mM INO 36.04 g myo-Inositol, ddH_2O to 1L and autoclave

20% Lactose 200g Lactose, ddH_2O to 600 ml and mix well to dissolve
Add ddH_2O to 1L. Microwave to warm and autoclave

20% Maltose 20g Maltose, ddH_2O to 100ml and ??

40% Glucose 400g Glucose, ddH_2O to 600 ml and mix well to dissolve
ddH_2O to 1L and autoclave

30% Galactose 300g Galactose, ddH_2O to 600 ml and mix well to dissolve
ddH_2O to 1L and autoclave

20% Raffinose 200g Raffinose, ddH_2O to 600 ml and mix well to dissolve
ddH_2O to 1L **and 0.2µ sterilize**

40% Sucrose 400g Sucrose, ddH_2O to 600 ml and mix well to dissolve
ddH_2O to 1L **and 0.2µ sterilize**

Amino acid powder

2.0g **Ade**	2.0g Gly	2.0g Pro
2.0g Ala	2.0g **His**	2.0g Ser
2.0g Arg	2.0g **Leu**	2.0g Thr
2.0g Asn	2.0g **Lys**	2.0g **Trp**
2.0g Asp	2.0g Ile	2.0g Tyr
2.0g Cys	2.0g Met	2.0g Val
2.0g Gln	0.2g paba	2.0g **Ura**
2.0g Glu	2.0g Phe	

-4aa; leave out His, Leu, Trp, Ura
-6aa; leave out His, Leu, Trp, Ura, Ade, Lys

Media for *E.coli*

(For solid medium add 20g Agar / L)

1 x LB: Standard *E.coli* growth medium. Not too rich (eg. 2xYT, below) and suitable for pretty much every application used in the lab (**eg.** amplifying plasmids, expressing recombinant proteins)

10g tryptone
5g yeast extract
10g NaCl
200µl 10M NaOH
dH_2O to 1 liter and autoclave

2 x YT: Richer *E.coli* growth medium. Suitable for many applications although density a bit much for *Qiagen* columns (assume >50% *E.coli* more / ml)

16g tryptone
10g yeast extract
5g NaCl
4ml 1M NaOH
dH_2O to 1 liter and autoclave

SOB: A base for SOC, which can improve the efficiency of *E.coli* transformation.

20g tryptone
5g yeast extract
0.58g NaCl
0.19g KCl
4ml 1M NaOH
dH_2O to 1 liter and autoclave.
Just before use add: 5ml 2M $MgCl_2$ / L

SOC: Add sterile glucose to 20mM (usually made in 5ml aliquots)

Media for *S.cerevisiae*

(For solid medium add 20g Agar / L)

-4aa medium 3g yeast nitrogen base (YNB)
10g ammonium sulfate
4g -4aa powder
2ml 200mM Inositol
dH_2O to 1 liter and autoclave
(Usually made as 2x but above is 1x. **Don't forget to add the desired sugar**)

-6aa medium 3g yeast nitrogen base (YNB)
10g ammonium sulfate
4g -6aa powder
2ml 200mM Inositol
dH_2O to 1 liter and autoclave
(Usually made as 2x but above is 1x. **Don't forget to add the desired sugar**)

1 x YEP (base for rich media for growth without selection but variable sugar source; Raffinose, Galactose or Glucose. **As sugars will have to be added later, usually made at a higher concentration, eg. 1.1 x YEP)**
20g Bacto-peptone
10g Yeast Extract
0.15g Tryptophan
dH_2O to 1 liter and autoclave

YPD (**YEP + Dextrose**; standard rich media for growth without selection)
20g Bacto-peptone
20g Glucose (dextrose)
10g Yeast Extract
0.15g Tryptophan
dH_2O to 1 liter and autoclave (not too long: sugars and $-NH_3$ react)

SPOR (Sporulation media; **for plates**)
10g Potassium Acetate
1.25g Yeast Extract
1g Glucose
20g Agar
ddH_2O to 1 liter, mix well and autoclave

Minimal media (Synthetic defined; **for Plates**)
500ml 2 x YNB
450ml ddH_2O
20g Agar, mix well and autoclave
50ml sterile 40% Glucose, mix well and pour

Media for *S. pombe*

(For solid medium add 20g Agar / L)

NOTE 3: Many additives for *S.cerevisiae* media can be used for culturing *S.pombe*. Fission yeast can be grown on YPD although they're not too happy about peptone.

YES: Yeast Extract with Supplements (the *S.pombe* version of YPD)
Standard rich media used for growth without selection.

30g glucose (30% w/v)
5g yeast extract (0.5% w/v)
Supplements: 225mg/L Adenine, histidine, leucine, uracil and lysine.HCL
dH_2O to 1 liter and autoclave

(If you want to see the red color associated with *ade6* mutations reduce the adenine to 10mg/L).

EMM2: Edinburgh Minimal Medium 2 (US Biologicals E2205, $58.90)
Thiamine-free formulation for nmt1 promoter studies. Note that Nitrogen Base contains enough Thiamine to repress the Nmt1 promoter.

3g/L	Potassium hydrogen phthallate	(14.7mM final)
2.2g/L	Na_2HPO_4	(15.5mM final)
5g/L	NH_4Cl	(93.5mM final)
20g/L	Glucose	(2% w/v)
20ml/L	**SALTS (x50)**	
1ml/L	**VITAMINS (x1000)**	
0.1ml/L	**MINERALS (x10,000)**	

SALTS (x 50)

52.5 g/l	$MgCl_2.6H_20$	(0.26 M)
0.735 mg/l	$CaCl_2.2H_20$	(4.99 mM)
50 g/l	KCl	(0.67 M)
2 g/l	Na_2SO_4	(14.l mM)

VITAMINS (x 1000)

1 g/l	pantothenic acid	(4.20 mM)
10 g/l	nicotinic acid	(8l.2 mM)
10 g/l	inositol	(55.5 mM)
10 mg/l	biotin	(40.8 μM)

MINERALS (x10,000)

5 g/l boric acid	(80.9 mM)
4 g/l $MnSO_4$	(23.7 mM)
4 g/l $ZnSO_4.7H2O$	(13.9 mM)
2 g/l $FeCl_2.6H2O$	(7.40 mM)
0.4 g/l molybdic acid	(2.47 mM)
1 g/l KI	(6.02 mM)
0.4 g/l $CuSO_4.5H2O$	(1.60 mM)
10 g/l citric acid	(47.6 mM)

COLOR CODE FOR YEAST PLATES

-Ura	purple
-His	orange
-Trp	red
-Leu	brown
-Lys	green
-Ade	yellow
-Ino	double green
+ glucose	black
+ galactose	blue
+ 3-AT	double yellow
+ 5-FOA	double black on both sides of code
YPD	double black
NGS	triple blue

COLOR CODE FOR *E.coli* PLATES

LB	double blue
+Amp	red
+Tet	green
+Kan	orange
+Cam	brown