Yeast Media (YM)

Distilled H20 : 1000 mL

Malt Extract : 3 g

Bacto Peptone : 5 g

Glucose (Dextrose) : 10 g

Agar : 20 g (10 g if using BTS brand)

Yeast Extract : 3 g

\*Autoclave

1mL chloramphenicol (100 mg/mL)

Yeast Media (YM) (update 10/5/20)

Distilled H20 : 1000 mL

YM agar mix : 41g

YM Broth Recipe

Same as above but omit Agar

R2A

18.1 g R2A Agar

400 mL of 40% Sucrose sol’n---160 g sucrose

600 mL DI H20

\*autoclave\*

1mL cycloheximide (100 mg/mL)- fungicide

\*.75 each

-80°C Cultures :

1. Sterilize working area
2. Fill Cryo Vial with 1.5 mL Glycerol Freezer Sol’n
   1. 15% Sucrose weight by volume, then enough glycerol to make the entire sol’n 15% glycerol
   2. EG- 150 mL glycerol in 850 mL DI H20 w/ 127.5 g sucrose, autoclaved
3. Add Colony to vial
4. Label vial with culture ID (also enter ID in excel sheet on Vannette Lab drive)
5. store in freezer overnight
6. Dilute colony in YM broth
7. Leave overnight
8. Add glycerol to make 25:75 (Glycerol:Broth)

1 L freezer stock sol’n

7.5 into 42.5 w/ 3.657

150 mL glycerol in 850 mL D1 H20 w/ 127.5 g sucrose

\*autoclave\* \*Make in small batches\*

15mL in 85mL D1 H2O w/ 12.75g sucrose

200 mL total

30mL glycerol + 170 mL di water + 25.5 g sucrose

100 mL total

15 mL glycerol + 85 mL di water + 12.75 g sucrose

50 mL total

7.5 ml glycerol + 42.5 mL di water + 7.375 g sucrose

NEW 9/15/17 NEAA 15% sugar liquid media artificial nectar:

990 mL DI H20 49.5

30g sucrose 1.5

60g glucose 3

60g fructose 3

1g peptone .05

\*autoclave\*

10mL MEM NEAA Sol'n (100x) .5

(^50mL)

NEW 9/15/17 High NEAA 15% sugar liquid media artificial nectar:

950 mL DI H20 47.5 14.25

30g sucrose 1.5 0.45

60g glucose 3 0.9

60g fructose 3 0.9

1g peptone .05 .015

\*autoclave\*

50mL MEM NEAA Sol'n (100x) 2.5 .75

(^50mL)(^15mL)

NEW 9/15/17 15% sugar liquid media artificial nectar agar:

990mL DI H20

30g sucrose

60g glucose

60g fructose

10g agar

1g peptone

\*autoclave\*

10mL MEM NEAA Sol'n (100x)

NEW 9/15/17 High Amino Acid 15% sugar liquid media artificial nectar agar:

990mL DI H20

30g sucrose

60g glucose

60g fructose

10g agar

1g peptone

\*autoclave\*

50mL MEM NEAA Sol'n (100x)

NEW 6/5/18 TSA media

1000mL DI H2O

15g tryptone

15g agar

5g soytone

5g NaCl

Optional: 50g fructose (for acinetobacter)

1mL cycloheximide (100mg/mL) (after autoclaving)- fungicide

Thrips TSB liquid media

950 mL DI H2O

15g tryptone

5g soytone

5g NaCl

75g fructose

75g sucrose

Stock Solutions

100mg/mL cycloheximide

1g of cycloheximide in 10mL methanol

Chloramphenicol is also dissolved in methanol

40% Sucrose

400g sucrose in 1L of milliQ water

50x TAE Electrophoresis Buffer

242 g tris, free base

18.61 g disodium EDTA

57.1 mL glacial acetic acid

ddi H2O to 1.0 L

Add the tris and EDTA to approximately 700 mL ddi H2O and stir until the tris and EDTA are dissolved. Add the acetic acid, then adjust the volume to 1.0 L (NOTE: always add acid into water, NEVER water into acid).

For 1 L of 1x TAE: Dilute 20 mL of the stock 50x TAE to 1.0 L (i.e., add 980 mL H2O)

For 5 L of 1x TAE: Dilute 100 mL of the stock 50x TAE for 5.0 L (i.e., add 4.9 L H2O)

A 1x TAE solution is 40 mM tris, 20 mM acetate, and 1 mM EDTA and typically has a pH around 8.6 (do not adjust).

Composition of SDA (for nematodes)

| Ingredients | In gm/L |
| --- | --- |
| Dextrose (Glucose) | 40 gm |
| Peptone | 10 gm |
| Agar | 15 gm |
| Distilled Water | 1000 ml |

New 11/12/2021

deMan, Rogosa, and Sharpe (MRS) + 2% fructose - for growing lactobacilli; make half batch if possible!

Distilled H2O : 1000 mL

MRS BROTH powder : 52 g (Remel™, catalog # R454062)

Fructose : 20 g

Agar (omit if making broth): 15 g

\*Stir

\*Autoclave

1 mL cycloheximide (100 mg/mL) fungicide

(Total mass of dissolved solids: 52+20+15+0.1 = 87.1g)

1% agarose gel, pre-cast with GelRed

150 mL 1x TAE buffer

1.5 g agarose

\*mix thoroughly\*

\*heat for 90 s in microwave, but every 30 s remove and mix\*

\*let cool 5 min\*

15 µL 10,000x GelRed stock

\*mix thoroughly\*

\*cast gel\*

Reference:<https://biotium.com/wp-content/uploads/2015/02/PI-41002-41003.pdf>