

SOLID MEDIA USED IN THIS COURSE

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24June90, 11July94, 18Aug95, 1July96, 11July97, 2July98, 2Aug00, 28June01, 1July04, 3Apr06, 15Aug07, 10July09
http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Media_Prep/Media_Common.htm

These media will be prepared for use in a variety of experiments during the quarter in Microbiology. Note the similarities and differences between them. You should become familiar with the purposes of each. Identify which are *selective* media, and which are *differential* media, and why. Note that all but the first are formulated as a complete powder, and therefore you **weigh out only the amount indicated in bold below the title** in a single weighing out the premixed powder. For specific directions on how to handle these powders, dissolve them, autoclave and pour plates, refer to *Solid Media Prep: 4% Glucose Nutrient Agar*. Create a page for the media your team makes up with actual weights of media you weigh out, and notes about the steps you and your team performed.

SET UP: Set up four stations around the room, each with assigned dry medium, balance, weighing container, spoon, grad cylinder, pot to bring to boil, thermometer, access to dH₂O. The number of 600 mL bottles to make for a class of 20 students is indicated.

NUTRIENT AGAR WITH 4% GLUCOSE

(For cultivating yeast and non-fastidious bacteria) (2 bottles = 1200 mL)

Dissolve the following in 600 mL dH₂O:

weigh out four items:

Nutrient Broth Powder	6.0 g
Contains: 2.25 g Beef Extract	
3.75 g Peptone	
NaCl	3.0 g
Glucose	24.0 g
Agar	9.0 g

LEVINE EMB AGAR (Selection and differentiation of *E. coli* and other Gm-bacteria) (5 bottles = 3000 mL)

weigh out 22.5 g/600 mL bottle

Ingredients per liter:

Peptone	10.0 g
Lactose	10.0 g
Dipotassium phosphate	2.0 g
Agar	15.0 g
Eosin Y	0.4 g
Methylene blue	0.065 g

PLATE COUNT AGAR (For examining milk and dairy products.) **NOTE:** Aliquot out 15 mL in 16x150 test tubes, cap and *then* autoclave.

weigh out 35.25 g/1500 mL

Ingredients per liter:

Tryptone	5.0 g
Yeast Extract	2.5 g
Glucose	1.0 g
Agar	15.0 g

MacCONKEY AGAR (Selection and differentiation of *E. coli* and other Gm-bacteria) (3 bottles = 1800 mL)

weigh out 30 g/600 mL bottle

Ingredients per liter:

Peptone	17.0 g
Proteose peptone	3.0 g
Lactose	10.0 g
Bile Salts No. 3	1.5 g
Sodium Chloride	5.0 g
Agar	13.5 g
Neutral Red	0.03 g
Crystal Violet	0.001 g

Cut here -----

MEDIA FOR PROPAGATION OF LIQUID CULTURES AND BACTERIOPHAGE

TRYPTONE AGAR (For growth of phage)*

grams/600 mL:

(Make 1 bottle for a class of 8)

tryptone	6.0 g
NaCl	0.5 g
1 M CaCl ₂ (2 mL/L medium)	1.2 mL
agar	6.6 g

TOP AGAR:*

For use in titering phage, and preparing a poured a lawn of bacteria in top agar.

grams/200 mL:

tryptone (1%)	2.0 g
KCl (0.5%)	1.0 g
agar (0.6%)	1.2 g
1 M CaCl ₂	0.4 mL

TRYPTIC SOY BROTH [TSB]

(Soybean-Casein Digest Medium, USP)

18 g/600 mL

For the cultivation of fastidious and non-fastidious organisms, culturing of coliphage.

Ingredients per liter:

Bacto-Tryptone	17.0 g
Bacto-Soytone	3.0 g
Bacto-Dextrose	2.5 g
Sodium Chloride	5.0 g
Dipotassium Phosphate	2.5 g

Mix, distribute 2 mL/13 x 100 mm test tube, cap and sterilize. Before use, melt in 100°C water, cool to 45°C. (Alternately, sterilize in bottle, melt just before use, aliquot out into 45°C capped sterile Wasserman tubes.)

* From Bob Kirchen at Carolina Biological, 2/7/95