

## Media Recipes

### 1. Mueller-Hinton agar

Commonly referred to as Mueller-Hinton agar, the current version of the medium is Mueller-Hinton II agar made by Becton, Dickinson and Company, as are all of the other bacterial media mentioned below.

The Clinical and Laboratory Standards Institute (3) specifies using Mueller-Hinton agar for the following reasons: acceptable batch-to-batch reproducibility; low in sulfonamide, trimethoprim, and tetracycline inhibitors; gives satisfactory growth for most organisms; and has been used successfully by many people.

#### Ingredients per liter

Beef extract	2.0 g
Acid hydrolysate of casein	17.5 g
Starch	1.5 g
Agar	17.0 g

Final pH 7.3 +/- 0.1

#### Preparation

Dissolve 38 g of agar in 1 liter of boiling purified water (purified water is obtained through reverse osmosis, distilled, or deionized) and mix thoroughly.

Stir the mixture and heat to boiling; allow it to boil for 1 minute to fully dissolve the powder.

Sterilize by autoclaving at 121°C for 15 minutes, do not overheat.

Pour into plates to a depth of 4 mm per plate, allow agar to cool and solidify.

Refrigerate plates if they will not be used that day. Wrap plates in plastic to delay drying. Plates can be kept for longer than 7 days.

### 2. Mueller-Hinton broth

Mueller-Hinton broth is used as a general medium to cultivate bacteria.

#### Ingredients per liter

Beef extract	3.0 g
Acid hydrolysate of casein	17.5 g
Starch	1.5 g

### **Preparation**

Suspend 22 g of the powder in 1 liter of purified water (as above). Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Sterilize by autoclaving at 121°C for 10 minutes. Do not overheat.

### **3. Nutrient broth**

#### **Ingredients per liter**

Pancreatic digest of gelatin	5.0 g
Beef extract	3.0 g

### **Preparation**

Suspend 8 g of the powder in 1 liter of purified water (as above). Mix thoroughly. Warm slightly if necessary to dissolve. Sterilize by autoclaving at 121°C for 15 minutes.

### **4. Trypticase soy agar**

#### **Ingredients per liter**

Pancreatic digest of casein	15.0 g
Papaic digest of soybean meal	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g

### **Preparation**

Suspend 40 g of powder in 1 liter of purified water (as above). Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Sterilize by autoclaving at 121°C for 15 minutes. Do not overheat. Cool until agar can be handled (at approximately 55°C) and pour into petri plates. Let solidify.

## Storing bacterial strains

There are several methods for storing bacteria. Much information is available in *Methods for General and Molecular Bacteriology* (1). Using two or more methods to preserve cultures enhances the chance that strains can be retained for a long period of time.

### 1. Long term storage on agar slants covered with sterile mineral oil and stored in the refrigerator

Mineral oil is available from the local pharmacy, and it can be sterilized by placing it in a heat resistant container with a heat resistant lid and baking in an oven at 180°C for 2 hours. Recover bacteria by removing a loopful of broth through the oil.

### 2. Anhydrous silica gel

The anhydrous silica gel method was published by Dr. John Lennox (2) and a quick description is presented here. It is a modification of the procedure presented in *Methods for General and Molecular Bacteriology* (1). In our hands, most cultures prepared according to his instructions and stored in the -20°C freezer survive for 5 years or even much longer. Materials required include glass vials and lids which can be sterilized in a dry oven (as described above), anhydrous silica gel (available from Aldrich, 21,442-6), instant nonfat dry milk (made up as a 7.5% solution and autoclaved to sterilize, make twice as much as desired due to coagulation of the milk proteins), slant cultures in stationary phase, freezer, pipettes (either regular or Pasteur pipettes) and a pipette bulb, dust mask and gloves (to protect from silica gel dust), and labeling materials. Prepare the silica gel by placing it into the small vials (borosilicate tubes with autoclavable screw caps, e.g., Kimble 60915-D, 2 dram vials). Many vials can be prepared, sterilized, and kept tightly closed in the freezer to be used when cultures need to be preserved. Vials must be cold when the bacteria are added since absorption of the liquid generates heat. Add 2 to 3 ml of sterile milk to each slant of bacteria, rinse the bacteria from the slant and then add them dropwise to the cold sterile silica gel. Add until the liquid is no longer absorbed. Allow to sit with lids slightly loose for 4 to 6 days, then tightly cap, label well, parafilm the lids shut, and place in the -20°C freezer. Do not use a frost-free freezer due to the freeze-thaw cycles.

### 3. Cooked meat medium

Cooked meat medium contains broth and small meat pellets. It works well as a medium to store anaerobes and facultative anaerobes, but has limited usefulness for strict aerobes. To prepare the medium, pour 1.25 grams of the medium into a screw-capped test tube and add 10 ml of purified water (see below). Allow the tube to stand for 10 minutes before autoclaving. Sterilize by autoclaving for 15 minutes at 121°C. Inoculate the tube allow bacteria to grow for 24 to 48 hours, then add 600 µl of sterile 80% glycerol (see below), mix well, cap loosely, place in the freezer until frozen, then cap tightly. Maintain in a non-frost-free freezer at

-20°C or -80°C. To revive the culture, scrape with a sterile wooden stick or loop while still frozen and touch to broth or solid media.

#### 4. Freezing media

A web search, literature search, or molecular biology book review will provide a large number of similar recipes for freezing bacteria. Some procedures utilize dimethylsulfoxide (DMSO) to create a flexible membrane, while others utilize glycerol.

One hundred percent glycerol is very anhydrous and does not autoclave adequately, nor does it mix well with water. If using glycerol for freezing, it is best to prepare a less anhydrous solution. To make eighty percent glycerol, add 20 ml of water to 80 ml of glycerol, mix, and autoclave at 121°C for 15 minutes in an autoclavable screw-capped bottle. The final concentration of glycerol in the frozen bacteria is usually between 5% and 15%.

Bacteria to be frozen should be fresh broth cultures, just at stationary phase. Cells are not usually frozen in media that contains antibiotics. Add the glycerol to the culture, mix, and aliquot into sterile microcentrifuge tubes. Seal and label the tubes. Freeze at -20°C in a non-frost-free freezer. After freezing at -20°C, cells can be moved to -80°C. To start a new culture, remove the tube from the freezer, keep it from thawing, open the tube, scrape with a sterile wooden stick or sterile toothpick, and inoculate a broth or agar culture. Consider making a backup set of the freezer stocks and storing them in another location.

DMSO is usually used at 7.5% concentration. If DMSO is used, it should be from a freshly opened bottle, a bottle resealed as it was flushed with gaseous argon or nitrogen (DMSO is relatively unstable), or aliquots from a freshly opened bottle that were frozen at -20°C or -70°C until use. As with glycerol, DMSO stabilizes the membrane of a cell. DMSO is added to stationary-phase cultures of bacteria to a final concentration of 7.5%, and the bacteria are sealed into microcentrifuge tubes and frozen. New stocks are started by scraping with a sterile wooden stick and inoculating into broth or agar media. DMSO is extremely volatile and even when used in a biological safety cabinet, the user will taste garlic, an indicator that DMSO is being absorbed through the gloves and skin.

#### References.

1. **Gerhardt, P., R. G. E. Murray, W. A. Wood, and N. R. Krieg (ed.).** 1994. *Methods for general and molecular bacteriology*. American Society for Microbiology, Washington, DC.
2. **Lennox, J. E.** 1977. Maintenance of bacterial cultures on anhydrous silica gel. *Am. Biol. Teacher* **39**:152–154.

3. **NCCLS.** 2000. Performance standards for antimicrobial disk susceptibility tests. Approved standard, 7th ed. NCCLS document M2-A7. NCCLS, Wayne, Pa. [www.nccls.org](http://www.nccls.org).