

BBL™ Chocolate II Agar with Bacitracin//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Japan

Cat. No. 251789 Prepared **I Plate™** Dishes – Pkg. of 20***BBL™ Chocolate II Agar with Pyridoxal**

Cat. No. 297259 Prepared Plates – Ctn. of 100*

BBL™ Chocolate II Agar//Martin-Lewis Agar

Cat. No. 297060 Prepared Bi-Plate Dishes – Pkg. of 20*

297245 Prepared Bi-Plate Dishes – Ctn. of 100*

BBL™ Chocolate II Agar//Modified Martin-Lewis Agar

Cat. No. 298513 Prepared Bi-Plate Dishes – Pkg. of 20*

298206 Prepared Bi-Plate Dishes – Ctn. of 100*

BBL™ Chocolate II Agar//Modified Thayer-Martin (MTM II) Agar**BS10** **CMPH** **MCM7**

Cat. No. 221623 Prepared Bi-Plate Dishes – Pkg. of 20*

BBL™ Chocolate II Agar//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

United States and Canada

Cat. No. 221302 Prepared **I Plate™** Dishes – Pkg. of 20*221303 Prepared **I Plate™** Dishes – Ctn. of 100*

Europe

Cat. No. 251302 Prepared **I Plate™** Dishes – Pkg. of 20*251303 Prepared **I Plate™** Dishes – Ctn. of 100***BBL™ Chocolate II Agar//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//MacConkey II Agar**Cat. No. 297140 Prepared **Y Plate™** Dishes – Pkg. of 20*299580 Prepared **Y Plate™** Dishes – Ctn. of 100*

*Store at 2-8°C.

Choline Assay Medium

Intended Use

Choline Assay Medium is used for determining choline concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimum growth of the test organism except the single essential vitamin to be determined.

User Quality Control

Identity Specifications

Difco™ Choline Assay Medium

Dehydrated Appearance: White, free-flowing, homogeneous.

Solution: 2.85% (single strength) solution, soluble in purified water upon boiling. Solution is colorless, clear, may have a slight precipitate.

Prepared Appearance: Colorless, clear, may have a slight precipitate.

Reaction of 2.85% Solution at 25°C: pH 5.5 ± 0.2

Cultural Response

Difco™ Choline Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Neurospora crassa* ATCC™ 9277 when prepared in single strength and supplemented with choline chloride. The medium should produce a standard curve when tested using a choline chloride reference standard at 0.0 to 25.0 µg per 10 mL. Incubate flasks with caps loosened at 25-30°C for 3 days. Measure the growth response gravimetrically – weight of mycelia versus micrograms of choline chloride standard.

Choline Assay Medium is a slight modification of the medium described by Horowitz and Beadle.¹ *Neurospora crassa* ATCC™ 9277 is the test organism used in this microbiological assay.

Principles of the Procedure

Choline Assay Medium is a choline-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *N. crassa* ATCC 9277. The addition of choline standard in specified increasing concentrations gives a growth response by this organism that can be measured gravimetrically.

Formula

Difco™ Choline Assay Medium

Approximate Formula* Per Liter

Sucrose	40.0	g
Ammonium Nitrate	2.0	g
Potassium Sodium Tartrate	11.4	g
Monopotassium Phosphate	2.0	g
Magnesium Sulfate	1.0	g
Sodium Chloride	0.2	g
Calcium Chloride	0.2	g
Ferrous Sulfate	1.1	mg
Zinc Sulfate	17.6	mg
Biotin	10.0	µg
Sodium Borate	700.0	µg
Ammonium Molybdate	500.0	µg
Cuprous Chloride	300.0	µg
Manganese Sulfate	110.0	µg

*Adjusted and/or supplemented as required to meet performance criteria.

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 5.7 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense 10 mL amounts into flasks, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust flask volume to 20 mL with purified water.
6. Autoclave at 121°C for 10 minutes.

Procedure

Remove 1 loop of spores from a 48-72 hour culture of *N. crassa* ATCC 9277 grown on Neurospora Culture Agar (per liter: Proteose Peptone No. 3, 5.0 g; Yeast Extract, 5.0 g; Maltose, 40.0 g; Agar, 15.0 g; pH 6.7 ± 0.2) and suspend it in 100 mL sterile saline. Add 1 drop of this spore suspension to each flask of medium. Incubate at 25-30°C for 3 days. At the end of the incubation period, steam the flask at 100°C for 5 minutes. Remove all the mycelium from the flask using a stiff wire needle or glass rod, press dry between paper towels, and roll into a small pellet. Dry the pellet at 100°C in a vacuum oven for 2 hours. (A glazed porcelain spot plate is convenient for handling the mycelium during drying and weighing.) Weigh to the nearest 0.5 mg. A standard curve is then constructed from the weights obtained, and the unknown determined by interpolation. In the assay for choline, 50 mL Erlenmeyer flasks containing a total volume of 10 mL each are used.

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using choline at levels of 0.0, 2.5, 5, 10, 15, 20 and 25 µg per assay flask (10 mL). The most effective assay range using Choline Assay Medium is between 2.5 and 30 µg choline.

The concentration of choline required for the preparation of the standard curve may be prepared by dissolving 0.5 g choline chloride in 1,000 mL purified water. This is the stock solution (500 µg per mL). Dilute the stock solution by adding 1 mL to 99 mL purified water. Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL of this diluted solution per flask. Prepare the stock solution fresh daily.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results to these procedures, all conditions of the assay must be followed precisely.

Reference

1. Horowitz and Beadle. 1943. J. Biol. Chem. 150:325.

Availability

Difco™ Choline Assay Medium

Cat. No. 246010 Dehydrated – 100 g*

*Store at 2-8°C.

Chopped Meat Carbohydrate Broth, PR II Chopped Meat Glucose Broth, PR II

Intended Use

Chopped Meat Carbohydrate Broth, PR II and Chopped Meat Glucose Broth, PR II are pre-reduced media used in the enrichment, cultivation and maintenance of anaerobic microorganisms, particularly obligate anaerobes.

Summary and Explanation

These media utilize Hungate's method for culturing anaerobic microorganisms outside of an anaerobic chamber.¹ The tubes provide a reduced medium in a self-contained, anaerobic culture

chamber sealed using a Hungate screw cap. The cap contains a butyl rubber septum stopper that permits inoculation and incubation outside an anaerobic chamber without exposing the medium to air.

They are recommended for subculture and enrichment of anaerobic isolates for chromatographic analysis and tests to determine proteolysis (meat digestion), spore formation, motility and toxin production, particularly by *Clostridium* species, and as a holding or stock culture maintenance medium.²⁻⁴