m-ENTEROCOCCUS AGAR (7544)

Intended Use

m-Enterococcus Agar is used for the selective isolation and enumeration of enterococci by membrane filtration.

Product Summary and Explanation

m-Enterococcus Agar was first described by Slanetz et al. for the enumeration of enterococci by the membrane filtration technique.[1] In 1957, Slanetx and Bartley modified this medium by adding triphenyltetrazolium chloride (TTC).[2] Increased recovery and larger colonies were obtained by incubating the inoculated membranes on the agar surface instead of on pads saturated with liquid medium. The membrane filtration method is simple to perform, does not require confirmation, and permits a direct count of enterococci in 48 hours. m-Enterococcus Agar is also referred to as m-Azide Agar.

The enterococcus group the are fecal streptococci and include *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. avium*.[3] Enterococci are differentiated from other streptococci by their ability to grow in 6.5% Sodium Chloride, at pH 9.6, and at 10°C and 45°C.[3] The presence of enterococci is a valuable bacterial indicator for determining the extend of fecal contamination of recreational surface waters.[3] m-Enterococcus Agar is used in standard methods for the detection of fecal streptococci using the membrane filtration technique.[3]

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal provides the nitrogen, minerals, and amino acids in m-Enterococcus Agar. Yeast Extract is the vitamin source and Dextrose supplies carbon. Dipotassium Phosphate acts as a buffer. Sodium Azide is the selective agent used to suppress the growth of gram-negative organisms. Agar is the solidifying agent. Triphenyl Tetrazolium Chloride (TTC) is the dye used as an indicator of bacterial growth. TTC is reduced to insoluble formazan inside the bacterial cell, resulting in the production of red colonies.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Soybean Meal	5 g
Yeast Extract	5 g
Dextrose	2 g
Dipotassium Phosphate	4 g
Sodium Azide	0.4 g
2,3,5-Triphenyl Tetrazolium Chloride	0.1 g
Agar	10 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful by inhalation and if swallowed. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 42 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE. Cool to 45 - 50°C and dispense.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light pinkish-beige.

Prepared Appearance: Prepared medium is light to medium pink-beige, and clear to slightly hazy.

Expected Cultural Response: Cultural response on m-Enterococcus Agar at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions
Enterococcus faecalis ATCC® 19433	growth	dark red colonies
Enterococcus faecalis ATCC® 29212	growth	dark red colonies
Enterococcus faecalis ATCC® 33186	growth	dark red colonies
Escherichia coli ATCC® 25922	inhibited	---
Staphylococcus aureus ATCC® 25923	Inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedures

Membrane filtration procedure

1. Follow the membrane filtration procedure as described in standard methods or by laboratory policy.[3]
2. Choose a sample size resulting in 20 - 60 colonies.
3. Transfer the filter to agar medium in a petri dish, avoiding air bubbles beneath the membrane.
4. Let plates stand for 30 minutes.
5. Invert plates and incubate at 35 ± 0.5°C for 48 hours.

Direct plating procedure

1. Inoculate medium with a specimen using the steak plate method.
2. Incubate plates at 35 ± 2°C for 24 - 48 hours.

Results[3]

Count all light and dark red colonies as enterococci. Count colonies using a fluorescent lamp and a magnifying lens.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

m-Entercoccus Agar	**Code No.**	**7544A**	**500 g**
		7544B	**2 kg**
		7544C	**10 kg**

References

1. **Slanetz, Bent, and Bartley**. 1955. Public Health Rep. **70**:67.
2. **Slanetz, and Bartley.** 1957. J. Bacteriol. **74**:591.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.