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, Slanetz 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 are fecal streptococci and include *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. avium*.[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 recommended for the detection of fecal streptococci using the membrane filtration technique for water testing.[3,4,5] The food industry also has applications for testing enterococci using m-Enterococcus Agar.[6,7]

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal provide 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. Skin irritation may be severe. Irritating to eyes, respiratory system, mucous membranes, 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 beige.

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

Expected Cultural Response: Cultural response on m-Enterococcus Agar incubated aerobically at 35 ± 0.5°C and examined for growth at 24 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
Enterococcus faecalis ATCC® 19433	10 - 100	Growth	Dark red to maroon colonies
Enterococcus faecalis ATCC® 29212	10 - 100	Growth	Dark red to maroon colonies
Enterococcus faecalis ATCC® 33186	10 - 100	Growth	Dark red to maroon colonies
Escherichia coli ATCC® 25922	~ 10^3	Inhibited	---
Staphylococcus aureus ATCC® 25923	~ 10^3	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 appropriate references or by laboratory policy.[3,4,5]
2. Choose a sample size resulting in the isolation of 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. Invert plates and incubate at 35 ± 0.5°C for 48 hours.

Direct plating procedure

1. If required, samples should be homogenized and diluted with saline to result in the isolation of 15 - 150 colonies.
2. Inoculate medium by spreading the sample evenly over the agar surface.
3. 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.

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.

Limitation of the Procedure

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

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.
4. **Environment Agency.** 2002. Microbiology of Drinking Water, Methods for examination of water and associated materials.
5. **ISO Standard for Water Quality.** Detection and enumeration of intestinal enterococci. Part 2. membrane filtration method.
6. **Burkwell, M.K. and P. A. Hartman.** 1964. Appl. Microbiol. **12:**18-23.
7. **Nordic Committee on Food Analysis.** 1968. Leaflet. 68.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

NEOGEN CORPORATION
620 Lesher Place, Lansing MI 48912
517/372-9200 • 800/783-3212 • fax: 800/875-8563
neogen-info@neogen.com • www.neogen.com