m-ENDO BROTH (7723)

Intended Use
m-Endo Broth is used for enumerating coliforms in water by the membrane filtration method.

Product Summary and Explanation
The coliform group are used as indicators of fecal pollution in water, for assessing the effectiveness of water treatment and disinfection, and for monitoring water quality. m-Endo Broth is used for selectively isolating coliform bacteria from water and other specimens using the membrane filtration technique.

m-Endo Broth is prepared according to the formula of Fifield and Schaufus.[1] It is recommended by the American Public Health Association (APHA) in standard total coliform membrane filtration procedure for testing water, wastewater, and foods.[2,3] The US Environmental Protection Agency specifies using m-Endo Broth in the total coliform methods for testing water using single-step, two-step and delayed incubation membrane filtration methods.[4,5]

Principles of the Procedure
Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Enzymatic Digest of Soy Flour provide nitrogen, carbon, and minerals in m-Endo Broth. Yeast Extract is a source of vitamins and trace elements to stimulate bacterial growth. Potassium Phosphates are the buffering agents. Sodium Chloride maintains the osmotic balance. Lactose serves as a carbohydrate source. Sodium Lauryl Sulfate and Sodium Deoxycholate are selective agents used to inhibit Gram-positive bacteria. Basic Fuchsin is a pH indicator. Sodium Sulfite is added to decolorize the Basic Fuchsin solution. Ethanol aids in the homogeneity of the solution and as a selective agent.

Lactose is the fermentable carbohydrate, and Lactose positive colonies exhibit a red color caused by the aldehyde reaction with the Sodium Sulfite and Basic Fuchsin. The development of a metallic sheen occurs when the organism produces aldehydes with the fermentation of Lactose. Lactose non-fermenting bacteria form clear, colorless colonies.

Formula / Liter
Lactose	12.5 g
Enzymatic Digest of Soy Flour	10.0 g
Enzymatic Digest of Animal Tissue	5.0 g
Enzymatic Digest of Casein	5.0 g
Sodium Chloride	5.0 g
Potassium Phosphate, dibasic	4.375 g
Potassium Phosphate, monobasic	1.375 g
Sodium Sulfite	2.1 g
Yeast Extract	1.5 g
Sodium Lauryl Sulfate	0.5 g
Sodium Deoxycholate	0.1 g
Basic Fuchsin	1.05 g

Final pH: 7.2 ± 0.2 at 25°C

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

Supplement
Non-denatured Ethanol, 20 mL

Precautions
1. For Laboratory Use.
2. TOXIC. Harmful if swallowed, inhaled, or absorbed through skin. May cause irritation to skin, eyes, and respiratory tract. Possible carcinogen.

Directions
1. Suspend 48 g of the medium in one liter of purified water containing 20 mL of non-denatured Ethanol.
2. Heat with frequent agitation and boil to completely dissolve the medium.
3. Avoid overheating. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and pink-purple to pink-red.

Prepared Appearance: Prepared medium is pink-purple to pink-red and hazy to opalescent with precipitate.

Expected Cultural Response: Cultural response in m-Endo Broth at 35 ± 2°C and examined for growth after 18 - 24 hours incubation.

Microorganism	Approx. inoculum (CFU)	Expected Results	
		Growth	Reaction
Escherichia coli ATCC® 25922	10 - 300	Good to excellent	Red colonies with green metallic sheen
Salmonella typhimurium ATCC® 14028	10 - 300	Good to excellent	Colorless to pink colonies
Staphylococcus aureus ATCC® 25923	300 - 1000	Inhibited	---

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

Test Procedure

Enrichment Method

1. Invert the dish and place an absorbent pad in the lid of a Petri dish.
2. Add 1.8 – 2.2 mL of Lauryl Tryptose Broth to each pad.
3. Place a membrane filter, through which the sample has passed, onto the pad containing LST Broth.
4. Incubate aerobically for 1.5 - 2 hours at 35°C.
5. Transfer the incubated membrane filter from the LST pad to a new pad that has 1.8 – 2.0 mL of m-Endo Broth added. Proceed following the Single-Step Method, Step 4.

Single Step Method

1. Place a membrane filter absorbent pad inside a sterile 60 mm Petri dish.
2. Add 1.8 – 2.0 mL m-Endo Broth to each pad.
3. Filter the sample through a membrane filter.
4. Place membrane filter top side up on the pad using a rolling motion to avoid entrapping air bubbles.
5. Incubate aerobically in an inverted position for 20 – 24 hours at 35 ± 0.5°C.
6. Observe the count all colonies that are red and have a metallic sheen.

Results

Following incubation, examine membrane filters for the presence of red colonies. All red colonies that have the characteristic metallic sheen are coliforms. The metallic green-gold sheen can cover all or part of the colony. Report the coliform density in terms of total coliforms / 100 mL. Lactose non-fermenting bacteria form clear, colorless colonies.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. 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 medium has changed from the original color. Expiry applies to medium in its intact container.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly.
2. If the inoculum is too heavy, the sheen may be suppressed.
3. Occasionally, noncoliform organisms may produce typical sheen colonies. Coliform organisms may also occasionally produce atypical colonies, including dark red or nucleated colonies without sheen.

Packaging

m-Endo Broth	**Code No.**	**7723A**	**500 g**
		7723B	**2 kg**
		7723C	**10 kg**

References

1. **Fifield, C. W., and C. P. Schaufus.** 1958. J. Am. Water Works Assoc. **50:**193-196.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
3. **Downes, F. P., and K. Ito** (eds.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
4. **Bordner, R., and J. Winter (eds.).** 1978. Microbiological methods for monitoring the environment, water, wastes. EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, OH.
5. **U. S. Environmental Protection Agency.** 1992. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, U. S. Environmental Protection Agency, Cincinnati, OH.

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.