m-TEC AGAR
SKU: 700003672, 700003673, 700003674
NCM0291

Intended Use

m-TEC Agar is used with urea for the isolation and enumeration of thermotolerant *Escherichia coli* from water using the membrane filtration technique in a laboratory setting. m-TEC Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Escherichia coli is used as an indicator of fecal pollution in water. Several tests are available for enumerating *E. coli* based on its ability to grow at elevated temperatures and indole production. The membrane filter procedure is recognized in Standard Methods as an alternate test procedure. m-TEC is an abbreviation for membrane thermotolerant *E. coli*.

In 1981, Dufour et al. developed a simple and accurate membrane filter technique for rapid enumeration of *E. coli*. In this study, the researchers were able to quantitate *E. coli* on m-TEC Agar within 24 hours without requiring subculture and identification of isolates. Dufour et al. recovered *E. coli* from marine, estuarine, and freshwater samples.

Typical Formulation

Enzymatic Digest of Animal Tissue	5.0 g/L
Yeast Extract	3.0 g/L
Lactose	10.0 g/L
Sodium Chloride	7.5 g/L
Potassium Phosphate	4.3 g/L
Sodium Lauryl Sulfate	0.2 g/L
Sodium Deoxycholate	0.1 g/L
Bromocresol Purple	0.08 g/L
Bromophenol Red	0.08 g/L
Agar	15.0 g/L

Final pH: 7.3 ± 0.2 at 25°C
Formula is adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 45.3 grams 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. Autoclave at 121°C for 15 minutes.
4. Dispense 4 – 5 mL amounts into 10 x 50 mm petri dishes, allow to solidify.

Urea Substrate

1. Combine 2 g urea and 10 mg phenol red in 100 mL purified water.
2. Adjust pH to 5.0 ± 0.3
3. Store at 2 - 8°C. Use within one week.

Note: Other methods may recommend an alternative pH. Prepare substrate according to recommended guidelines.

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Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light greyish to green beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and dark purple.

Expected Cultural Response: Cultural response on m-TEC Agar using the membrane filtration technique and incubated aerobically at 44.5°C. Cultures were examined for growth after 22 ± 2 hours. Filters were transferred to a pad saturated with urea substrate and held at room temperature for 15 to 20 minutes. Urease – negative, thermotolerant *E. coli* colonies are yellow, yellow-green, to yellow-brown.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Urease Reaction*
Enterococcus faecalis ATCC® 29212	~10^3	Markedly suppressed to Complete inhibition	N/A
Escherichia coli ATCC® 8739	10 - 100	Good to excellent	Negative (Yellow, yellow-green to yellow-brown colonies)
Escherichia coli ATCC® 35150	10 - 100	Good to excellent	Negative (Yellow, yellow-green to yellow-brown colonies)
Escherichia coli ATCC® 35218	10 - 100	Good to excellent	Negative (Yellow, yellow-green to yellow-brown colonies)
Proteus vulgaris ATCC® 13315	100 - 300	Markedly suppressed to Complete inhibition	N/A
Pseudomonas aeruginosa ATCC® 27853	~10^3	Markedly suppressed to Complete inhibition	Colorless

The organisms listed are the minimum that should be used for quality control testing.
*Different membrane brands may affect the colony color

Test Procedure

1. Follow membrane filter procedure described in Standard Methods.
2. Incubate inoculated plates for 2 hours at 35°C to resuscitate injured cells.
3. Transfer plates to a 44.5 ± 0.5°C water bath or incubator and incubate for 22 ± 2 hours.
4. Place a 50 mm absorbent pad into petri dish. Add approximately 2 mL of urea substrate to pad (pad should be saturated with urea substrate without any standing liquid in petri dish).
5. Transfer countable filters to pads saturated with urea substrate.
6. After 15 - 20 minutes, count all yellow, yellow-green, to yellow-brown colonies with the aid of a stereoscopic microscope.

Results

Yellow to yellow-brown colonies (urease negative) may be presumptively identified as *E. coli*.

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.

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Limitations of the Procedure

1. The 35°C incubation step is required to resuscitate stressed organisms. The 44.5°C incubation temperature is required to inhibit non-thermotolerant organisms.
2. The urease test is required to presumptively identify *E. coli*.
3. Choose a water sample size that will result in 20 - 80 colonies per filter. Higher counts may not provide accurate urease test results.
4. Do not trap air bubbles underneath the filter.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. 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.

References

1. Mara, D. D. 1973. A single medium for the rapid detection of *Escherichia coli* at 44°C. J. Hyg. 71:783-785.
2. Pugsley, A. P., L. J. Evision, and A. James. 1973. A simple technique for the differentiation of *Escherichia coli* in water examination. Water RES. 7:1431-1437.
3. Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.). 2017. Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, Washington, D.C.
4. Dufour, A. P., E. R. Strickland, and V. J. Cabelli. 1981. Membrane filter method for enumerating *Escherichia coli*. Appl. Environ. Microbiol. 41:1152-1158.
5. Dufour, A. P., and V. J. Cabelli. 1975. Membrane filter procedure for enumerating the component genera of the coliform group in seawater. Appl. Microbiol. 29:826-833.
6. 1996 Annual Book of ASTM Standards, Water and Environmental Technology (PCN: 01-11-296-16). ASTM, West Conshohocken, PA.

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