m-ENDO LES AGAR

- For in vitro use only - Catalogue No. PE54 & PE55

Our m-Endo LES Agar is a selective membrane filtration medium used for the enumeration of coliform organisms from water.

In 1904, Endo reported the development of a culture medium that allowed for the differentiation of lactose fermenters from lactose non-fermenters. McCarthy, Delaney, and Grasso modified Endo's formulation and using the membrane filter technique developed a two-step process that gave superior recovery of coliform bacteria from water. The initial step, a resuscitation and enrichment step, involved exposing the filter to Lauryl Tryptose Broth for several hours; the filter was then placed onto a modified Endo agar, which is now known as the LES (Lawrence Experimental Station) formulation.

The coliform group is defined as aerobic or facultative anaerobic, gram-negative, non-spore forming bacteria able to produce acid and gas from lactose fermentation at 35°C. On m-Endo LES Agar, coliforms appear as red colonies with a metallic green sheen. This unique coloration is due to the production of aldehyde from the fermentation of lactose; the aldehyde can liberate fuchsin from the colorless Schiff's reagent (fuchsin-sodium sulfite) making colonies appear red. In the case of *E.coli*, this reaction is so intense that the fuchsin crystallizes out giving the colonies a metallic green sheen. The selective agents contained in the medium, sodium desoxycholate and sodium lauryl sulfate help to inhibit non-coliforms.

The m-Endo LES formulation is recommended by the American Public Health Association (APHA) for the analysis of total coliforms in drinking water and bottled water. It is not suitable for testing waters with high turbidity or wastewaters containing toxic metals or toxic organic compounds.

Formula per Litre of Medium

Pancreatic Digest of Animal Tissue	7.5 g
Casein Hydrolysate	3.7 g
Meat Peptone	3.7 g
Yeast Extract	1.2 g
Lactose	9.4 g
Potassium Phosphate (Dibasic)	3.3 g
Potassium Phosphate (Monobasic)	1.0 g
Sodium Chloride	3.7 g
Sodium Desoxycholate	0.1 g
Sodium Lauryl Sulfate	0.05 g
Sodium Sulfite	1.6 g
Basic Fuchsin	0.8 g
Agar	15.0 g
Ethanol	20.0 mL

pH 7.2 ± 0.2

Recommended Procedure (APHA)
(Refer to APHA literature for a more details)

1. Allow m-Endo LES Agar plates to warm to room temperature prior to use.
2. Place a sterile absorbent pad in the lid of the m-Endo plate. Add 2.0-mL of Lauryl Tryptose Broth to the pad. Once the pad is saturated decant any excess liquid.
3. Using an appropriate sterile filtration unit, filter the water sample. (Typical sample size is 100-mL)
4. Aseptically, place the membrane filter onto the saturated absorbent pad. Cover and incubate agar-side up for 1.5 to 2 hours at 35°C.
5. Once the incubation period has elapsed, aseptically transfer the membrane filter to the m-Endo LES Agar. Slowly roll the membrane filter onto the surface of the agar to minimize air bubbles. If patches of air are

observed carefully reposition the filter.

6. Incubate plate in an inverted position, agar side up, for 22 ± 2 hours.
7. Enumerate typical and atypical coliform colonies using a dissecting microscope or other suitable optical device.

Interpretation of Results

A typical coliform colony has a pink to red color with a metallic green sheen; the sheen may cover the entire colony or may only appear in the center or on the periphery.

Colorless, white, or blue colonies are classified as non-coliforms. It should be noted that some colonies will appear pink or red but lack the characteristic metallic sheen. These colonies are classified as atypical coliforms and need to be verified through further testing. Verification can be achieved through lactose fermentation tests or commercially available rapid tests.

For enumeration purposes, the APHA recommends that membrane filters with 20 to 80 coliforms and not more than 200 colonies of all types be counted. If the total number of colonies exceeds 200 or if confluent growth is observed the sample is classified as TNTC. If at least one detectable coliform colony can be verified then the TNTC sample can be classified as a coliform positive sample. For TNTC samples request a new sample and select a more appropriate filtration volume. Coliform density should be calculated and reported as follows:

$$\text{(Total) coliforms/100mL} = \frac{\text{Coliform colonies counted} \times 100}{\text{mL sample filtered}}$$

- *APHA recommends verifying both typical and atypical colony types for coliform bacteria*

- *m-Endo LES Agar is sensitive to light and drying therefore do not expose the medium unnecessarily during storage or use. Do not use plates if they darken or develop a surface sheen*

- *The membrane filtration technique may be inappropriate for testing high turbidity waters or where large numbers of non-coliform bacteria are present*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Escherichia coli ATCC 25922	Growth, red with metallic green sheen
Enterobacter aerogenes ATCC 12453	Growth, red with or w/o metallic green sheen
Staphylococcus aureus ATCC 25923	Inhibition

Storage and Shelf Life

Our m-Endo LES Agar should be protected from light and stored at 4 to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 2 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
PE54	m-Endo Agar [Standard 15x100-mm plate]	10/pkg
PE55	m-Endo Agar [Membrane 15x60-mm plate]	10/pkg

References

1. Endo S. Uber ein verfahren zum nachweis der typhusbacillen. Centr Bakt, Abt 1, Orig 1904; 35:109-10.

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3. McCarthy JA, Delaney JE, Grasso RJ. Measuring coliforms in water. Water Sewage Works 1961; 108:238.
4. MacFaddin JF. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
5. Calabrese JP, Bisonnette GM. Appl Env Microbiol 1990; 56:3558-64.
6. Eaton AD, Clesceri LS, Greenberg AE, Eds. Standard methods for the examination of water and wastewater. 19th ed. Washington, DC: APHA, 1999.

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