85766 m-Endo Agar LES (Membrane Endo Agar LES) NutriSelect® Plus

For the enumeration of coliforms in water using the Membrane filter technique. This method is more reliable an precice than MPN multiple test. It follows the two step membrane filter procedure using Lauryl Sulfate Broth (Cat. No. 17349) as a preliminary enrichment, resulting in higher coliform counts.

Composition:

Ingredients	Grams/Litre
Yeast extract	1.2
Casein hydrolysate	3.7
Peptone from meat	3.7
Tryptose	7.5
Lactose	9.4
di-potassium hydrogen phosphate	3.3
Potassium hydrogen phosphate	1.0
Sodium Chloride	3.7
Sodium deoxycholate	0.1
Sodium lauryl sulfate	0.05
Sodium sulfite	1.6
Pararosanilin (fuchsin)	0.8
Agar	15.0

Final pH 7.2 +/- 0.2 at 25°C

Store dehydrated powder below 30°C in a tightly closed container and use freshly prepared media.Protect from moisture and light by keeping container in a low humidity environment. Use before expiry date on the label.

Appearance(color):	Faint purple to light purple and pink, free flowing powder
Gelling:	Firm, comparable with 1.5% Agar gel
Color and Clarity:	Red to very dark red coloured clear to slightly opalescent gel forms in Petri plates

Directions:
Suspend 51 g in 1 litre of distilled water containing 20 ml of ethanol 96 %. Boil to dissolve completely. Do not autoclave! Cool to 45-50°C and dispense 4 ml amounts into 50-60 mm Petri dishes and allow to solidify. The agar should be at least 1.5mm depth. Do not expose plates to direct sunlight.

Principle and Interpretation:
In 1904, Endo reported the development of a culture medium that allowed for the differentiation of lactose fermenters from lactose non-fermenters(1). M-Endo agar, LES is a modification of the original medium, formulated by McCarthy, Delaney, and Grass by using the membrane filter technique resulting into a two-step process that gave better recovery of coliform bacteria from water. The initial step, after filtration, is a resuscitation and enrichment step, involved exposing the filter to Lauryl Tryptose Broth for 1.5-2 hours; the filter was then placed onto a modified Endo agar, which is now known as the LES (Lawrence Experimental Station) formulation(2). This medium is recommended by APHA for the analysis of colifom in drinking and bottled water (3, 4).

Experimental Procedure:

1. Invert plate and place membrane filter pad (top side up, avoid air bubbles) in the lid and add 1.8-2.0 ml Lauryl Sulfate Broth to each pad. Remove any excess liquid.
2. Incubate at 35 °C for 1.5 to 2.0 hours in a humid atmosphere.
3. Transfer the filter - again top side up - to the surface of the agar. Avoid entrapment of air.
4. Incubate inverted plates at 35 °C ± 0.5 °C for 20 to 24 hours aerobically.

The Coliform bacteria are defined as facultatively anaerobic, Gram-negative, non-spore-forming rods that ferment lactose vigorously to acid and gas at 35 ± 2 °C within 24 or 48 h.On m-Endo LES Agar, coliforms appear as pink to red colonies with a metallic green sheen. This unique coloration is due to the production of aldehyde from the fermentation of lactose; the aldehyde liberates fuchsin from the colorless Schiff's reagent (fuchsin-sodium sulfite) making colonies appear pink to 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 present in the media, sodium desoxycholate and sodium lauryl sulfate helps to inhibit non-coliforms. Organisms do not ferment lactose stay colorless.

Casein enzymic hydrolysate, tryptose, peptic digest of animal tissue and yeast extract provide essential nutrients especially nitrogenous for the coliforms. Lactose is the fermentable carbohydrate. Sodium chloride is for the osmotic balance. Sodium sulphite, sodium deoxycholate and basic fuchsin inhibit the growth of gram-positive organisms. Phosphates buffer the medium.

Cultural characteristics observed after an incubation of 20-24 hrs at 35 -37°C

Organisms (ATCC/WDCM)	Inoculum (CFU)	Growth	Color of colony (on membrane filter)
Escherichia coli (25922/ 00013)	50-100	++/+++	Pink with metallic sheen
Enterobacter aerogenes (13048/00175)	50-100	++/+++	Pink to red (they may have sheen)
Klebsiella pneumoniae (13883/00097)	50-100	++/+++	Pink to red
Pseudomonas aeruginosa (27853/-)	50-100	+++	Pink
Salmonella Typhi (6539/-)	50-100	+++	Colorless to light pink
Staphylococcus aureus (25923/00034)	$\geq 10^4$	-	
Salmonella Typhimurium (14028/00031)	50-100	+++	Colorless to very light pink

References:

1. Endo S., 1904, Zentralbl. Bakteriol., Abt. 1, Orig.35:109-110
2. McCarthy J. A., Delaney J. E. and Grasso R., 1961, Water and Sewage Works, 108:238
3. Eaton A. D., Clesceri L. S. and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
4. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.