39734 Membrane Lactose Glucuronide Agar (MLGA; M-Lauryl Sulfate Chromogen Agar)

A chromogenic medium for the differentiation and enumeration of *Escherichia coli* and other coliforms, which simplifies the membrane filtration technique for *E. oli* and coliforms by reducing the number of filtration stages required from two to one and by reducing the need for further confirmation steps. This medium is described in the Environment Agency's report of UK, 'Methods for Examination of Waters and Associated Material - The Microbiology of Drinking Water (2002) - Part 4 - Methods for the isolation and enumeration of coliform bacteria and *Escherichia coli* (including *E. coli* O157:H7)'.

Composition:

Ingredients	Grams/Litre
Peptone	40.0
Yeast extract	6.0
Lactose	30.0
Phenol red	0.2
Sodium lauryl sulphate	1.0
Sodium pyruvate	0.5
X-Glucuronide (BCIG)	0.2
Agar	10.0
Final pH (at 25°C) 7.4 ± 0.2	

Prepared media may be stored at temperatures below 8°C for up to one week, protected against dehydration and from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:
Suspend 88g in 1 litre of distilled water. Mix well and sterilize by autoclaving at 121°C for 15 minutes. Cool the medium to 50°C and pour into sterile petri dishes.

Methode/Technique:
For the analysis of treated liquids 100 ml should be filtered through filter (Φ 47mm, pore size 0.45µm) to get a reasonable number of colonies (20-80 colonies). In case a high number of bacteria is expected in the liquid a smaller volume or a dilution should be filtered. The membrane filter is placed on the Membrane Lactose Glucuronide Agar plate, ensure that no air bubbles are trapped between the membrane filter and the medium. The recommended incubation procedure is first 30°C for 4 hours for the high recovery rate and then for 14 hours.at 37°C. May an early indication after 12 hours is possible, but the plates should be incubated for at least 18 hours to get a reliable result.
Read the results within 15 minutes after removing from incubator as the yellow coloration may change on cooling and standing. *E. coli* gives green colonies while other coliforms are yellow. Count the green and yellow colonies and calculate the coliforms per mililiter, expressed in cfu/ml (colony forming units per ml).

Principle and Interpretation:
A chromogenic edium for detection, differentiation and enumeration of coliforms in water and other samples. The medium is recommended from “The Environment Agency” with a single membrane filtration technique (1).

Peptone and yeast extract provide amino acids, vitamines and other complex substances. Sodium lauryl sulphate inhibits gram-positive organisms. Sodium pyruvate protects injured cells, helps recovery of coliforms and enhances growth. Lactose is a carbohydrate source which can be fermented by the coliform bacteria, by using β-galactosidase, and the pH-indicator phenol red indicates that by changing to a yellow color (yellow colonies). X-Glucuronide is a chromogen substrate which can be cleaved by β-glucuronidase present in the *E. coli*. This results in a blue colony but in combination with the lactose fermentation it ends with a green colony.

Normally no further confirmation step is needed as the specificity of the green colonies being *E. coli* is very high. But if a further confirmation step is preferred there would be plenty of further tests like the Indole test etc.
Blue colonies are may be lactose-negative *E. coli*, but are more commonly strains of *Aeromonas*. Blue colonies should be classed as presumptive coliform bacteria and further testing is needed.
Seldom yellow colonies may confirm as *E. coli* (as some strains do not express β-glucuronidase or appear negative when first isolated) and green colonies may not confirm as *E. coli* but may, nevertheless, confirm as oliform bacteria.
Some rare species of *Bacillus* and *Staphylococcus* may grow on membrane lactose glucuronide agar producing yellow colonies. They can be easily identified by colony characteristics on MacConkey Agar, and by Gram staining.

Cultural characteristics after 24 hours at 37°C

Organism (ATCC)	Growth	Colour of Colony
Escherichia coli (25922)	+++	green
Klebsiella pneumoniae (13883)	++	yellow, mucoid
Enterobacter aerogenes (13048)	+++	yellow
Pseudomonas aeruginosa (27853)	+++	pink
Staphylococcus aureus (25923)	-	-
Bacillus subtilis (6633)	-	-
Salmonella serotype Enteritidis (13076)	++	pink

References:

1. The Environment Agency, Methods for Examination of Waters and Associated Material, The Microbiology of Drinking Water (2002)
2. D.P. Sartory, L. Howard, A medium detecting beta-glucuronidase for the simultaneous membrane filtration enumeration of Escherichia coli and coliforms from drinking water. Letters in Applied Microbiology, 15, 273-276 (1992)
3. Standing Committee of Analysts, Evaluation trials for two media for the simultaneous detection and enumeration of Escherichia coli and coliform organisms, Methods for the Examination of Waters and Associated Materials, in this series, Environment Agency (1998)

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.