

m-LGA (7721) (Membrane Lactose Glucuronide Agar)

Intended Use

m-LGA (Membrane Lactose Glucuronide Agar) is used for the differentiation and enumeration of *Escherichia coli* and other coliforms through the membrane filtration technique.

Product Summary and Explanation

Escherichia coli is used as an indicator of fecal pollution in water, for assessing the effectiveness of water treatment and disinfection, and for monitoring water quality.^{1,2,3} Several tests are available for enumerating *E. coli* based on its ability to grow at elevated temperatures and indole production.^{1,2} The membrane filter procedure is recognized in Standard Methods for the Examination of Water and Wastewater³ as an alternate test procedure.

m-LGA is enhanced by the addition of a chromogenic agent, X-Glucuronide, detecting glucuronidase activity. The presence of the enzyme β -D-glucuronidase differentiates most *E. coli* spp. from other coliforms, and is the same enzyme used in the MUG reactions.⁴ X-Glucuronide reacts slightly different and when released into the medium is insoluble, accumulating within the cell.

Principles of the Procedure

Enzymatic Digest of Gelatin provides nitrogen, amino acids, and essential minerals in m-LGA. Yeast Extract contains vitamins, and Lactose is the carbon energy source. Phenol Red is a pH indicator, detecting Lactose fermentation and producing yellow colonies when acid is produced. Sodium Pyruvate protects injured cells to enhance the growth of coliforms. Sodium Lauryl Sulfate is the selective agent to inhibit Gram-positive organisms. *E. coli* absorbs the chromogenic substrate, 5-bromo-4-chloro-3-indolyl- β -D-glucuronide, X-Glucuronide. The enzyme β -glucuronidase splits the bond between the chromophore 5-bromo-4-chloro-3-indolyl and the β -D-glucuronide. The released chromophore is colored and a blue color accumulates within the cells. This results in a blue colony, but in combination with the lactose fermentation, the result is a green colony. Ordinarily, no further confirmation step is needed as the specificity of *E. coli* as green colonies is very high. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	40.0 g
Yeast Extract.....	6.0 g
Lactose	30.0 g
Phenol Red	0.2 g
Sodium Lauryl Sulfate.....	1.0 g
Sodium Pyruvate.....	0.5 g
X-Glucuronide	0.2 g
Agar	10.0 g

Final pH: 7.4 \pm 0.2 at 25°C

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

Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful when in contact with skin and if swallowed. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 88 g 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.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige to red-tan with or without dark particles.

Prepared Appearance: Prepared medium is clear to trace hazy and bright red.

Expected Cultural Response: Cultural response on m-LGA incubated aerobically at $30 \pm 2^\circ\text{C}$ for 4 hours and then $35 \pm 2^\circ\text{C}$ for 14 – 20 hours and examined for growth at a total incubation of 18 – 24 hours.

Microorganism	Approx. Inoculum (CFU)	Growth	Color
		<i>Escherichia coli</i> ATCC® 25922	10 - 300
<i>Enterobacter aerogenes</i> ATCC® 13048	10 - 300	Growth	Yellow colonies
<i>Pseudomonas aeruginosa</i> ATCC® 27853	10 - 300	Growth	Pink colonies
<i>Bacillus subtilis</i> ATCC® 6633	10 - 300	Inhibited	---

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

Test Procedure

1. The volume and dilution of water filtered should be chosen to give the number of colonies counted on the membrane as 20 – 80. For treated waters 100 mL should be filtered. For polluted waters a smaller volume or a diluted sample should be used.
2. Following the sample filtration, place the membrane filter onto a prepared plate of m-LGA ensuring that no-air bubbles are trapped under the membrane.
3. Incubate inoculated plates at 30°C for 4 hours, then at 37°C for 14 - 20 hours. For an early indication of results, plates may be examined for colonies at 12 hours but must be re-incubated for the full 18 hours.
4. For the complete procedure, refer to The Environment Agency Report of UK.⁵

Results

Coliforms are lactose-positive, producing yellow colonies. *Escherichia coli* is both lactose-positive and possesses glucuronidase, producing green colonies.⁵ Count all yellow and green colonies. Yellow colonies are presumptive non-*Escherichia coli* coliform bacteria and green colonies are *Escherichia coli*. Under normal circumstances, no further confirmation step is needed as the specificity of green colonies being *E. coli* is very high. If a confirmation step is preferred, refer to appropriate references.³ The combined count can be regarded as the total number of coliform bacteria. Results are indicated in colony forming units per volume of sample, cfu/ml. Read and record results within 15 minutes after removing from incubator as the yellow color may change on cooling and standing.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C . Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to expiration date stamped on the container. Expiry applies to medium in its intact container and stored as directed.

Limitations of the Procedure

1. Blue colonies may be lactose-negative *E. coli*, but are more commonly strains of *Aeromonas* spp. Blue colonies should be considered presumptive coliform bacteria and further testing is needed.
2. On a rare occasion, yellow colonies may confirm as *E. coli*, as some strains do not express glucuronidase, and green colonies may not confirm as *E. coli* but may, nevertheless, confirm as coliform bacteria.
3. Rare species of *Bacillus* and *Staphylococcus* may grow on m-LGA producing yellow colonies. They can easily be identified by colony characteristics on MacConkey Agar and by a Gram stain.

Packaging

m-LGA	Code No.	7721A	500 g
		7721B	2 kg
		7721C	10 kg

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. Evison, and A. James.** 1973. A simple technique for the differentiation of *Escherichia coli* in water examination. Water RES. **7**:1431-1437.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **Feng, P. C. S., and P. A. Hartmann.** 1982. Appl. Environ. Microbiol. **43**:1320-1329.
5. **The Environment Agency Report of UK.** 2002. The Microbiology of Drinking Water, Part 4, Methods for the isolation and enumeration of coliform bacteria and *Escherichia coli*.

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.