

BMA AGAR (6903) **(Buffered MUG Agar)**

Intended Use

BMA (Buffered Mug Agar) is used with LMG Agar and the ISO-GRID[®] and/or NEO-GRID[™] Membrane Filtration System for the direct enumeration of Glucuronidase-Positive *Escherichia coli*.

Product Summary and Explanation

Buffered MUG Agar is formulated to take advantage of the ability of 4-methylumbelliferone to fluoresce at an alkaline pH under long wave (365 nm) ultraviolet light. The formula consists of phosphate buffered saline modified to contain 4-methylumbelliferyl- β -D-glucuronide (100 mg/L) and 1.5% (w/v) agar. The formula enables rapid detection of β -glucuronidase activity in bacterial colonies previously exposed to sodium glucuronate, an inducer of β -glucuronidase production by *E. coli*.

Buffered MUG Agar is recommended to be used with Lactose Monensin Glucuronate (LMG) Agar for detection and enumeration of *E. coli* from all foods using the ISO-GRID and/or NEO-GRID Membrane Filtration Method.^{1,2}

Principles of the Procedure

Sodium Phosphate, Monobasic and Dibasic, buffer the final pH of the medium to 7.4 ± 0.2 . Sodium Chloride maintains the osmotic environment. 4-Methylumbelliferyl- β -D-Glucuronide (MUG) is a fluorogenic substrate for β -glucuronidase. *E. coli* β -glucuronidase attacks MUG to yield 4-methylumbelliferone, a water soluble compound producing a blue-white fluorescence under long wave (365 nm) ultraviolet light. Agar is the solidifying agent.

Formula / Liter

Sodium Phosphate, Dibasic.....	8.23 g
Sodium Phosphate, Monobasic	1.2 g
Sodium Chloride	5 g
4-Methylumbelliferyl- β -D-Glucuronide	0.1 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

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

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 29.5 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 light to medium beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and very light to light beige.

Expected Cultural Response: Cultural response of quality control organisms were inoculated on LMG Agar using the ISO-GRID and/or NEO-GRID Membrane Filtration System method, and incubated for 24 ± 2 hours at 35°C. After incubation, the filters were transferred to Buffered MUG Agar, incubated at 35 ± 1.0°C for 2 - 5 hours and examined under long wave (366 nm) ultraviolet light.

Microorganism	Approx Inoculum (CFU)	Expected Results / Fluorescence
<i>Escherichia coli</i> ATCC 25922	10 - 300	Fluorescence
<i>Citrobacter freundii</i> ATCC 8090	10 - 300	No fluorescence

Test Procedure

For sample details refer to the ISO-GRID Methods Manual and NEO-GRID Protocols.

1. After obtaining the Total Coliform Count on LMG Agar, use sterile forceps to transfer the ISO-GRID membrane filter, grid side up, to a BMA plate.
2. Incubate the BMA plate for 2 - 5 hours at 35 - 37°C.
3. Examine the membrane under long-wave (366nm) ultraviolet light in a dark room.

Results

E. coli colonies fluoresce bright blue-white. Colonies fluorescing any other color, and colonies that are only pin-point in size are not *E. coli*. Ignore any colonies with a pale yellow fluorescence.

If positive colonies are present, count the number of squares containing *E. coli*. Convert the number of squares to the corresponding MPN and calculate the confirmed *E. coli* MPN, using the methods described in the ISO-GRID Methods Manual.

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 by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Approximately 6% of the *E. coli* strains including the enterohemorrhagic (O157:H7) strains are glucuronidase negative and will be missed on this medium.³ Alternate procedures must be used for these organisms. Please consult appropriate references.⁴

Packaging

BMA Agar (Buffered MUG Agar)

Code No.

6903A

500 g

References

1. **Entis, P.** 1989. Hydrophobic grid membrane filter/MUG method for total coliform and *Escherichia coli* enumeration in foods: Collaborative study. J. AOAC. **72**:936-950.
2. **Entis, P., and P. Boleszczuk.** 1990. Direct enumeration of coliforms and *Escherichia coli* by hydrophobic grid membrane filter in 24 hours using MUG. J. Food Prot. **53**:948-952.
3. **Hartman, P.** 1988. MUG (glucuronidase) test for *Escherichia coli* in food and water. Proc. 5th International Symposium on Rapid Methods and Automation in Microbiology. Florence, Italy.
4. **Hitchins, A., P. Hartman, and E. Todd.** 1992. Coliforms-*Escherichia coli* and its toxins, p. 325-369. In C. Vanderzant and D. Splittstoesser (eds.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association.

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.