

MI Broth, 2 mL (6510)

Intended Use

Ampouled MI Broth, 2 mL is used for the detection of Total Coliforms and *E. coli* in water testing using the membrane filtration method.

Product Summary and Explanation

Ampouled MI Broth, 2 mL is a prepared, ready to use medium for membrane filtration testing for the detection of Total Coliforms and *E. coli*, following the EPA approved method for drinking water.¹ This test method describes a sensitive and differential membrane filter medium for the concurrent detection and enumeration of Total Coliforms and *E. coli* in water samples in 24 hours or less, dependent upon the enzyme activity. Total Coliforms include species that may inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation, and water. They are usually found in fecally-polluted water and are often associated with disease outbreaks. The Total Coliform test is the primary indicator of bacteriological quality for potable water, distribution system water, and public water supplies because it is a larger measure of pollution than the Fecal Coliform Test.^{2,3}

This EPA Method (1604)¹ is used mainly by certified drinking water laboratories for the microbial analysis of potable water. The bacterial colonies that grow on the plate are inspected for the presence of a blue color from the breakdown of IBDG by the *E. coli* enzyme β -glucuronidase and fluorescence under longwave ultraviolet light (366 nm) from the breakdown of MUGal by the Total Coliform enzyme β -galactosidase.¹

Principles of the Procedure

Enzymatic Digest of Animal Tissue is the nitrogen source in MI Broth. Yeast Extract supplies vitamins, and Lactose is the carbon energy source. Sodium Chloride maintains the osmotic balance of the medium, while Potassium Phosphates are used as buffers. Sodium Lauryl Sulfate and Sodium Deoxycholate are selective agents to inhibit non-coliform bacteria and Gram-positive organisms. The two enzyme substrates are 4-Methylumbelliferyl- β -D-galactopyranoside (MUG, fluorogen) and Indoxyl- β -D-glucuronide, (chromogen) used for the detection of the enzymes β -galactosidase and β -glucuronidase for Total Coliforms and *E. coli* respectively. *E. coli* produces the enzyme glucuronidase that hydrolyzes MUG to yield a fluorogenic product that is detectable under long-wave (366 nm) UV light.

Medium Composition:

| | <u>Per Liter</u> |
|--|------------------|
| Enzymatic Digest of Animal Tissue..... | 5.0 g |
| Yeast Extract..... | 3.0 g |
| Lactose | 1.0 g |
| Sodium Chloride | 7.5 g |
| Potassium Phosphate, Monobasic | 1.0 g |
| Potassium Phosphate, Dibasic | 3.3 g |
| Sodium Lauryl Sulfate..... | 0.2 g |
| Sodium Deoxycholate | 0.1 g |
| Indoxyl-Beta-d-Glucuronide Cyclohexylammonium Salt (IBDG)..... | 0.32 g |
| 4-Methylumbelliferyl-Beta-d-Galactopyranoside (MUGal)..... | 0.1 g |
| Cefsulodin | 0.005 g |

Physical Characteristics

Appearance of medium: Trace to slightly hazy, light amber
 pH at 25°C: 7.05 \pm 0.2

Test Procedure

Preparation

1. Assemble the manifold or filtration flask that will supply the vacuum source, complete with rubber stopper.
2. Using a gentle twisting motion, secure the funnel adapter into the rubber stopper.
3. Using the same gentle twisting motion, secure the Neogen Filter onto the funnel adapter.

Ampouled Media

Filtration Procedure

1. Remove filtration cover and carefully pour the sample onto the filter.
2. Apply vacuum just long enough to pull the sample through the filter (if using a manifold, open only one valve at a time.)
3. Rinse the inside walls of the filter funnel with approximately 20 mL of sterile buffered solution. Apply vacuum just long enough to pull the solution through the filter, and turn off vacuum. Note: This step is optional if only water is being tested.
4. Briefly remove the filter and its funnel adapter from the rubber stopper to release any remaining vacuum pressure, and then re-secure into the stopper.
5. Add the MI-Broth onto the top of the filter. When doing so, be careful not to touch the filter with the tip of the ampoule.
6. Very briefly apply vacuum so that the media does not pool on top of the filter, and is visible underneath the filter. (Note: The media has been soaked correctly into the filter if there is a small pocket of air around the bottom port. The filter should be moist, but not oversaturated or dry.)
7. Remove and appropriately discard the plastic funnel. Place the filtration system cover over the filter/base assembly converting the unit to a Petri dish for sample incubation.
8. Remove the filter from the funnel adapter, and place a plug on the open bottom port.
9. Place the filtration plate into the incubator inverted so that the cover is on the bottom, and incubate at $35 \pm 2^\circ\text{C}$. Read and record results after 18 – 24 hours.
10. Dispose of test materials in accordance with all applicable local, state, and federal regulations.

Expected Cultural Response:

Sterile water was added to sterile filtration units and inoculated with the cultures listed below. The inoculum was filtered followed by the ampouled MI Broth and the filtration housing removed. Plates were incubated aerobically at $35 \pm 2^\circ\text{C}$ and examined for growth at 18 – 24 hours.

| Microorganism | Approx. Inoculum (CFU) | Expected Results | |
|--|------------------------------|--|----------------------|
| | | Growth | Fluorescence |
| Uninoculated Media | NA | No Growth | NA |
| <i>Aeromonas hydrophila</i> ATCC 7966 | 10 - 300 | Suppressed to Inhibited | Negative |
| <i>Citrobacter freundii</i> ATCC 8090 | 10 - 300 | $\geq 85\%$ recovery, white to clear colonies | Negative or Positive |
| <i>Enterobacter aerogenes</i> ATCC 13048 | 10 - 300 | $\geq 85\%$ recovery, yellow to clear colonies | Positive |
| <i>Enterococcus faecalis</i> ATCC 29212 | 10 - 300 | Suppressed to Inhibited | Negative |
| <i>Escherichia coli</i> ATCC 25922 | 10 - 300 | $\geq 85\%$ recovery, blue colonies | Positive |
| <i>Escherichia coli</i> ATCC 11775 | 10 - 300 | $\geq 85\%$ recovery, blue colonies | Positive |
| <i>Klebsiella pneumonia</i> ATCC 13883 | 10 - 300 | $\geq 85\%$ recovery, yellow to clear colonies | Positive |
| <i>Proteus mirabilis</i> ATCC 12453 | 10 - 300 | Suppressed to Inhibited | Negative |
| <i>Pseudomonas aeruginosa</i> ATCC 10145 | 10 - 300 | Suppressed to Inhibited | Negative |

Results¹

In this method, Total Coliforms are those bacteria that produce fluorescent colonies upon exposure to long wave ultraviolet light and primary culturing on MI Broth. The fluorescent colonies can be completely blue-white (Total Coliforms other than *E. coli*) or blue-green (*E. coli*) in color or fluorescent halos may be observed around the edges of the blue-green *E. coli* colonies. In addition, non-fluorescent blue colonies, which rarely occur, are added to the total count because the fluorescence is masked by the blue color from the breakdown of IBDG.

Count all blue colonies on each MI plate under normal/ambient light, and record the results. This is the *E. coli* count. Positive results that occur in less than 24 hours are valid, but the results cannot be recorded as negative until the 24 hour incubation period is complete.

Expose each MI plate to long wave ultraviolet light (366 nm), and count all fluorescent colonies (blue/green fluorescent *E. coli*, blue/white fluorescent Total Coliforms other than *E. coli*, and blue/green with fluorescent edges (also *E. coli*). Record the data.

Add any blue, non-fluorescent colonies found on the same plate to the Total Coliform count.

Storage

Store Ampouled MI Broth, 2 mL at 2 - 8 °C.

Expiration

Refer to expiration date printed on the front of the box container.

Limitations of the Procedure¹

1. Analyze sample as soon as possible after collection.
2. Samples containing colloidal or suspended particulate material can clog the membrane filter, thereby prevent filtration, or cause spreading of bacterial colonies which could interfere with colony identification.
3. Tiny, flat or peaked pinpoint blue colonies may be due to species other than *E. coli*. These colonies occur occasionally in low numbers and should be excluded from the count of the *E. coli* colonies, which are much larger in size (1 – 3 mm in diameter).
4. Bright green, fluorescent, non-blue colonies, observed along with the typical blue/white or blue-green fluorescent Total Coliform colonies may be species other than coliforms. These colonies, which generally occur in low numbers and can usually be distinguished from Total Coliform colonies.

Packaging

| | | | |
|------------------------------|-----------------|-------------|------------------|
| MI Broth, 2 mL | Code No. | 6510 | Box of 50 |
| Neogen Filter "White" | Code No. | 6550 | Box of 50 |
| Neogen Filter "Black" | Code No. | 6555 | Box of 50 |

References

1. **U. S. Environmental Protection Agency.** 2002. Method 1604: Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI Medium). EPA- 821-R-02-024. Office of Water. Washington DC, 20460.
2. **U. S. Environmental Protection Agency.** 2007. R9 Laboratory SOP1101. Membrane filtration coliform analysis.
3. **U. S. Environmental Protection Agency.** 1992. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, U. S. Environmental Protection Agency, Cincinnati, OH.

Technical Information

Contact Neogen Corporation for Technical Service or questions involving Ampouled Media at (517)372-9200 or (800)-234-5333 or fax us at (517)372-2006.