

## m-TGE Broth, 2 mL (6515)

### Intended Use

**Ampouled m-TGE Broth, 2 mL** is used for the determination of bacterial counts using membrane filtration method.

### Product Summary and Explanation

Ampouled m-TGE Broth, 2 mL is a prepared, ready to use medium for membrane filtration testing. m-TGE Broth, an abbreviation for membrane Tryptone Glucose Extract, is a nonselective nutrient medium for the determination of bacterial counts by the membrane filtration method. It was originally developed in the 1930's by Bower and Hucker for use in dairy products.<sup>1</sup> In 1948, the American Public Health Association (APHA) adopted Tryptone Glucose Extract Agar for use in testing milk and dairy products.<sup>2</sup>

The Heterotrophic Plate Count (HPC), formerly known as standard plate count, is used to count non-specific bacteria in water. Currently, APHA specifies Tryptone Glucose Extract Agar for the heterotrophic plate count procedure in testing bottled water.<sup>3</sup> This method can be applied to the analysis of drinking water under EPA's Surface Water Treatment Rules (40 CFR 141.74) and reagent water used for laboratory testing.<sup>3</sup> The method may be used to monitor changes in the bacteriological quality of finished water throughout a distribution system, thus giving an indication of the effectiveness of chlorination.<sup>4</sup> This method is also listed in Standard Methods for Water and Wastewater, Method 9215D,<sup>3</sup> EPA's Microbial Methods for monitoring the Environment,<sup>5</sup> and EPA's Manual for the Certification of Laboratories Analyzing Drinking Water.<sup>6</sup>

### Principles of the Procedure

Enzymatic Digest of Casein and Beef Extract provide the nitrogen, minerals, vitamins, and amino acids in m-TGE Broth. Dextrose supplies carbon as an energy source.

### Medium Composition:

### Per Liter

Enzymatic Digest of Casein .....	10 g
Beef Extract .....	6 g
Dextrose.....	2 g

Final pH: 7.0 ± 0.2 at 25°C

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

### Physical Characteristics

Appearance of medium: Clear, yellow to gold  
pH at 25°C: 7.0 ± 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.

#### 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.

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5. Add m-TGE 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 24 - 48 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 m-TGE Broth and the filtration housing removed. Plates were incubated aerobically at  $35 \pm 2^{\circ}\text{C}$  and examined for growth at 24 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Uninoculated Media	NA	No Growth
<i>Bacillus subtilis</i> ATCC 9372	10 - 300	$\geq 85\%$ recovery
<i>Escherichia coli</i> ATCC 25922	10 - 300	$\geq 85\%$ recovery
<i>Micrococcus luteus</i> ATCC 9341	10 - 300	$\geq 85\%$ recovery
<i>Saccharomyces cerevisiae</i> ATCC 9763	10 - 300	$\geq 85\%$ recovery
<i>Staphylococcus aureus</i> ATCC 25923	10 - 300	$\geq 85\%$ recovery

### Results

Count all colonies that grow on the surface of the membrane and record.

### Storage

Store Ampouled m-TGE Broth, 2 mL at  $2 - 8^{\circ}\text{C}$ .

### Expiration

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

### Limitations of the Procedure

1. Analyze sample as soon as possible after collection.
2. Because bacteria found in bottled water demonstrate a prolonged lag phase during adaptation to growth on m-TGE medium, extended incubation beyond 48 hours may be required.

### Packaging

m-Endo Broth, 2 mL	Code No.	6500	Box of 50
Neogen Filter "White"	Code No.	6550	Box of 50
Neogen Filter "Black"	Code No.	6555	Box of 50

### References

1. **Slanetz, Bent, and Bartley.** 1955. Public Health Rep. **70**:67.
2. **American Public Health Association.** 1948. Standard methods for the examination of dairy products, 9th ed. American Public Health Association, New York, N.Y
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1998. Standard methods for the examination of water and wastewater, 20<sup>th</sup> ed. American Public Health Association, Washington, D.C.

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4. **Bordner, R., and J. Winter (eds.).** 1978. Microbiological methods for monitoring the environment, water, wastes. EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, OH.
5. **U. S. Environmental Protection Agency.** 2007. R9 Laboratory SO1108. Heterotrophic plate count for bacteria in water.
6. **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.
7. **Kim and Feng.** 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

### **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.