



m-TGE with Indicator Broth, 2 mL

Product Number: 6516



Intended Use

Ampouled m-TGE with Indicator Broth, 2 mL is used for the determination of bacterial counts using the membrane filtration method in a laboratory setting. It is not intended for use in the diagnosis of disease or other conditions in humans.

Product Summary

Ampouled m-TGE with Indicator Broth, 2 mL is a prepared, ready-to-use medium for membrane filtration testing. m-TGE with Indicator 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. In 1948, the American Public Health Association (APHA) adopted Tryptone Glucose Extract Agar for use in testing milk and dairy products. A redox dye indicator has been added to the m-TGE to aid in visual detection of growing bacteria.

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. This method can be applied to the analysis of drinking water under the EPA's Surface Water Treatment Rules (40 CFR 141.74) and reagent water used for laboratory testing. 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. This method is also listed in the Standard Methods for Water and Wastewater, Method 9215D, EPA's Microbial Methods for Monitoring the Environment, and EPA's manual for the Certification of Laboratories Analyzing Drinking Water.

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. The indicator is added to develop color and provide contrast for colonies growing on top of a white filter.

Medium Composition

Enzymatic digest of casein	10 g
Beef extract	6 g
Dextrose	2 g
Indicator	0.1 g

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. Properly sterilize the manifold, rubber stoppers, and plastic adapters. Disinfect the rubber stoppers and plastic adapters by soaking in 10% bleach for 10–15 minutes, then rinse with sterile water.
3. Using a gentle twisting motion, secure the funnel adapter into the rubber stopper.
4. Using the same gentle twisting motion, secure the NEOGEN® filter onto the funnel adapter.

Filtration Procedure

1. Remove the filtration cover and carefully pour the sample onto the filter.
2. Apply the 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 the vacuum just long enough to pull the solution through the filter and turn off the 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 m-TGE with Indicator 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 the 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 of 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. Note: Be careful not to touch the bottom port of monitor with hands or gloves to avoid possible contamination).
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 the test materials in accordance with all applicable local, state, and federal regulations.





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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 addition of an m-TGE with Indicator Broth 2 mL ampoule and the filtration housing was removed. Plates were incubated aerobically at 35 ± 2 °C and examined for growth at 22–48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Uninoculated media	N/A	No growth
<i>Bacillus subtilis</i> ATCC 9372	10–300	≥ 85% recovery
<i>Escherichia coli</i> ATCC 25922	10–300	≥ 85% recovery
<i>Micrococcus luteus</i> ATCC 9341	10–300	≥ 85% recovery
<i>Staphylococcus aureus</i> ATCC 25923	10–300	≥ 85% recovery

Results: Count all colonies that grow on the surface of the membrane and record. Most colonies will develop a pink to red coloration, though some will be yellow to orange.

Storage

Store m-TGE with Indicator Broth, 2 mL at 2–8 °C protected from light.

Expiration

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

Limitations of the Procedure

1. Analyze the sample as soon as possible after collection.
2. Because bacteria found in bottled water demonstrates a prolonged lag phase during adaptation to growth on m-TGE with Indicator medium, extended incubation beyond 48 hours may be required.
3. Count only the colonies that develop on the surface of the filter. Any color development below the filter, including the pad or the bottom port, should not be counted.
4. Investigate sterilization techniques of the manifold, rubber stoppers, and plastic adapters if color develops below the filter, including the pad, become a reoccurring issue.
5. Countable plates have <300 CFU. If a sample has a high amount of growth, performance may be impacted due to limited availability of the media and selective components. The sample should be retested with dilution.

NEOGEN Items		
6516	m-TGE with Indicator Broth, 2 mL	Box of 50
6550	NEOGEN® filter — white	Box of 50

References

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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.
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