m-*E.coli*/Coliform Broth Ampoule, 2 mL

700002412 | 6511

Ampouled m-*E.coli*/Coliform Broth, 2 mL is a prepared, ready-to-use medium for membrane filtration testing for the detection of Total Coliforms and *E.coli*. 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 within 24 hours or less.

Total Coliforms includes species that may exist naturally in soil, water, and vegetation but also thrive in the intestines of warm-blooded animals. They are often associated with disease outbreaks. *E.coli* is a species of Coliform, not typically found to self-propagate outside of the intestinal track of humans and other animals. As such, it can be used as a primary indicator of a system breach and potential fecal coliform and pathogen contamination.

Medium Composition	
Enzymatic digest of casein	1.0 g
Yeast extraction	2.0 g
Sodium chloride	5.0 g
Monosodium phosphate dihydrate	2.2 g
Disodium phosphate anhydrous	2.7 g
Sodium pyruvate	1.0 g
D-Sorbitol	1.0 g
L-Tryptophan	1.0 g
Secondary alcohol ethyloxylate surfactant	0.15 g
Chromogenic mix	0.65 g

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

Physical Characteristics
Appearance of medium: Clear, light amber, no precipitate
pH at 25°C: 6.80 ± 0.2

Intended Use

Ampouled m-*E.coli*/Coliform Broth, 2 mL is used for the detection of Total Coliforms and *E. coli* in water testing 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 or animals.

Principles of the Procedure

Enzymatic digest of casein is the nitrogen source in m-*E.coli*/Coliform Broth. Yeast extract supplies vitamins, and D-Sorbitol is the carbon energy source. Sodium chloride maintains the osmotic balance of the medium, while sodium phosphates are used as buffers. The surfactant is the selective agent to inhibit non-coliform bacteria and gram-positive organisms. The chromogenic mix is converted by the enzymes β-glucuronidase and β-galactosidase for simultaneous detection of Total Coliforms and *E.coli*. Total Coliforms produce the enzyme galactosidase that yield pink to purplish colonies. *E.coli* produce glucuronidase that hydrolyses the chromogen to develop violet to dark blue colonies.

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 filter unit, such as Neogen® filter 700003884 | 6550, 700003886 | 6556, or 700003885 | 6555 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-*E.coli*/Coliform 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 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. Incubate at 36 ± 2°C. Read and record results after 21–24 hours.
10. Dispose of the test materials in accordance with all applicable local, state, and federal regulations.

Expected Cultural Response

The inoculum was filtered followed by the ampouled m-*E.coli*/Coliform Broth and the filtration housing removed. Plates were incubated aerobically at 36 ± 2°C and examined for growth at 21–24 hours. Incubation extended to 24 hours if color reactions are delayed. Sterile water was added to sterile filtration units and inoculated with the cultures listed below.

Microorganisms	Inoculum (CFU)	Recovery	Reaction
Uninoculated media	N/A	No growth	N/A
Escherichia coli — ATCC 8739*	~10–100	≥85% recovery**	Violet to blue colonies
Escherichia coli — ATCC 25922	~10–100	≥85% recovery**	Medium to dark blue colonies
Enterobacter aerogenes — ATCC 13048	~10–100	≥85% recovery**	Pink to purplish pink colonies
Citrobacter freundii — ATCC 43864	~10–100	≥85% recovery**	Pink to purplish pink colonies
Pseudomonas aeruginosa — ATCC 10145	~1,000	Suppressed to inhibited	Beige to yellow colonies, where recovered
Enterococcus faecalis — ATCC 19433	~10,000	Complete inhibition	N/A

*Escherichia coli strains that are weak β-galactosidase producers may be recovered which do not elicit a dark blue reaction.
**As compared to tryptic soy agar results.

Note: In comparison to a previously approved lot

Results

Following the incubation, filters should be observed for bacterial colony growth under ambient light. *E.coli* colonies will appear as medium to dark blue colonies and other coliforms will appear as pink to light purplish pink colonies. Report and record counts as follows:

E. coli Count

Count all medium to dark blue colonies on each m-*E.coli*/Coliform plate under normal/ambient light; record the results. This is the *E.coli* count. **Note:** 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.

Total Coliform Count

Count all *E.coli* and other coliforms on each m-*E.coli*/Coliform plate under normal/ambient light conditions; record the results. This is the Total Coliform count. **Note:** 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.

E. coli = Medium to violet to dark blue

Coliforms (other than *E.coli*) = Pink/light purplish pink

Storage

Store the Ampouled m-*E.coli*/Coliform Broth, 2 mL at 2–8°C keep it protected from the 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. Samples containing colloidal or suspended particulate material can clog the membrane filter, thereby preventing filtration, or causing the spreading of bacterial colonies which could interfere with colony identification.
3. For best results when testing syrup or beverages (other than water), be sure to give the filter unit an additional rinse with sterile PBS Buffer (after filtering the syrup or beverage sample) and filter the buffer.
4. Some non-target species may develop a reddish color if incubated beyond 24 hours; this color is not indicative of a coliform.
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		
6511	m-*E.coli*/Coliform Broth, 2 mL	Box of 50
6550	Neogen filter — white	Box of 50

References

U. S. Environmental Protection Agency. 2007. R9 Laboratory SOP1101. Membrane filtration coliform analysis.

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.

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