Rapid m-Green Selective Broth, 2 mL

Product Number: 6506

Intended Use

Ampouled Rapid m-Green Selective Broth, 2 mL is used for the detection of yeast and fungi in beverages by the membrane filtration method in a laboratory setting. Ampouled Rapid m-Green Selective Broth, 2 mL is not intended for use in the diagnosis of disease or other conditions in humans.

Product Summary and Explanation

Ampouled Rapid m-Green Selective Broth, 2 mL is a prepared, ready to use medium for membrane filtration testing. It is an improved modification of the formula, m-Green Yeast and Fungi Broth. Rapid m-Green Selective Broth contains selective agents inhibiting potential aciduric bacteriological contaminants and offering isolated yeast and mold counts. It is also rich in nutrients, providing an environment for excellent fungal growth in 3 days or less.

Fungi have been found in potable water and on the inner surface of distribution system pipes. They can survive water treatment, or they enter the system after treatment and remain viable.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and amino acids in Rapid m-Green Selective Broth. Yeast Extract is the vitamin source. Dextrose is an energy source for metabolism of fungi. Potassium Phosphate is a buffering agent. Magnesium Sulfate, Thiamine, and Diastase (a mixture containing amylolytic (starch) enzymes) provide essential ions, minerals, and nutrients. The selective mixture inhibits aciduric bacterial growth.

Medium Composition	Per Liter
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue	5 g
Yeast Extract	9 g
Dextrose	50 g
Magnesium Sulfate	2.1 g
Potassium Phosphate	2 g
Diastase	0.05 g
Thiamine	0.05 g
Dye Indicator	0.026 g
Selective Mixture	0.300 g

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

Physical Characteristics

Appearance of medium:	Clear, dark green.
pH at 25°C:	6.9 ± 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 manifold, rubber stoppers, and plastic adapters. Disinfect 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 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 Rapid m-Green Selective 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 the 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 (be careful not to touch the bottom port of monitor with hands or gloves to avoid possible contamination).
9. Place the NEOGEN filter into the incubator inverted so that the cover is on the bottom and incubate at 25–27°C. Read and record results after 2 to 3 days (see note 3 under Limitations of the Procedure).
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 ampoule media and the filtration housing removed. Plates were incubated aerobically at 26.0 ± 1.0°C and examined for growth at 24–72 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Uninoculated Media	NA	No Growth
Aspergillus brasiliensis (niger) ATCC 16404	~50–300	≥ 85% recovery
Candida albicans ATCC 10231	~50–300	≥ 85% recovery
Penicillium roquefortii ATCC 10110	~50–300	≥ 85% recovery
Saccharomyces cerevisiae ATCC 9763	~50–300	≥ 85% recovery
Trichophyton mentagrophytes ATCC 9533	~50–300	≥ 85% recovery
Escherichia coli ATCC 25922	~1,000	Inhibited

Results

All colonies growing on the surface of the membrane should be counted. Mold colonies generally appear white and filamentous to green or black and powdery due to spore development. Yeast colonies are generally cream colored and opaque. However, environmental strains of yeasts and molds may produce pigments or pigmented spores that appear pink, orange, red or other colors.

Storage

Store Ampouled Rapid m-Green Selective Broth, 2 mL at 2–8 °C, protected from light.

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. Samples containing colloidal or suspended particulate material can clog the membrane filter, thereby prevent filtration, or cause spreading of microbial colonies which could interfere with colony identification.
3. To establish that no growth is recovered, filters should be held up to 3 days or as established by internal validation of the procedure. To establish that a positive test result is complete, internally validate the optimum time frame for holding the filters. To do this test the recommended quality control organisms listed under Expected Cultural Response. Species growth rates can vary, so the optimum time frame may vary as well.
4. Incubating this test at 25–27°C is essential to some fungi recovery; incubating out side of this range can compromise recovery.
5. Countable plates have <300 CFU. Iif 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.

Packaging

Rapid m-Green Selective Broth, 2 mL	**Code No.**	**6506**	**Box of 50**
NEOGEN Filter “Black”	Code No.	6555	Box of 50
NEOGEN Filter “Black, 0.8 µm”	Code No.	6556	Box of 50

References

Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 1998. Standard methods for the examination of water and wastewater, 20th 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.

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