m-Green Yeast and Fungi Broth, 2 mL (6505)

Intended Use

Ampouled m-Green Yeast and Fungi Broth, 2 mL is used for the detection of yeast and fungi in beverages by the membrane filtration method.

Product Summary and Explanation

Ampouled m-Green Yeast and Fungi Broth, 2 mL is a prepared, ready to use medium for membrane filtration testing. m-Green Yeast and Mold Broth is an improved modification of the formula, m-Yeast and Mold Broth. The addition of Bromocresol Green aids in identification as it is absorbed into fungal colonies. m-Green Yeast and Fungi Broth is a relatively complex formula compared to other media used for the isolation of fungi and yeast. It is also rich in nutrients, providing an environment for excellent fungal growth.

Fungi have been found in potable water and on the inner surface of distribution system pipes.[1] They can survive water treatment or they enter the system after treatment and remain viable.[1]

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and amino acids in m-Green Yeast and Fungi 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. Metabolic by-products from fungal growth diffuse into the surrounding medium, lowering the pH which inhibits bacterial growth, and producing an acid reaction that causes residual Bromocresol Green to change to yellow, which is an acid reaction. The colonies are green due to diffusion of Bromcresol Green into the colonies.

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
Bromcresol Green	0.026 g

Final pH: 4.6 ± 0.2 at 25°C

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

Physical Characteristics

Appearance of medium:	Clear, dark green.
pH at 25ºC:	4.6 ± 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.
5. Add m-Green Yeast and Mold 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.
9. Place the Neogen filter into the incubator inverted so that the cover is on the bottom, and incubate at 25 - 30ºC. Read and record results after 2 – 7 days.
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 ampoulized m-Green Yeast and Fungi Broth and the filtration housing removed. Plates were incubated aerobically at 25 - 30ºC and examined for growth at 2 – 7 days.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Uninoculated Media	NA	No Growth
Aspergillus 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

Results®
All colonies growing on the surface of the membrane should be counted. Mold colonies generally appear white with a green tint and are filamentous. Yeast colonies are cream colored and opaque.

Storage
Store Ampouled m-Green and Yeast Mold Broth, 2 mL at 2 - 8 ºC.

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.

Packaging
m-Green Yeast and Fungi Broth, 2 mL	**Code No.**	**6505**	**Box of 50**
Neogen Filter "White"	**Code No.**	**6550**	**Box of 50**
Neogen Filter "Black"	**Code No.**	**6555**	**Box of 50**

References

1. **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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