

Instructions for Use

MODIFIED LISTERIA ENRICHMENT BROTH

Cat. no. U167	Modified Listeria Enrichment Broth, 500ml Polycarbonate Bottle, 225ml	10 bottles/box
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INTENDED USE

Hardy Diagnostics Modified Listeria Broth is used for selectively enriching *Listeria monocytogenes* from raw and pasteurized milk and milk products according to the International Dairy Federation (IDF) and the U.S. FDA Bacteriological Analytical Manual (BAM).^(5,6)

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

First described by Murray, Webb, and Swann, *Listeria monocytogenes* is a widespread problem in public health and the food industries.⁽⁷⁾ This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women. The first reported food-borne outbreak of listeriosis was in 1985.⁽⁵⁾ Since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.⁽⁹⁾

Implicated vehicles of transmission include turkey frankfurters, coleslaw, pasteurized milk, Mexican-style and other soft cheeses, pate and pickled pork tongue.⁽⁵⁾ The organism has been isolated from commercial dairy and other food processing plants.⁽⁶⁾ It is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.

Listeria spp. grow over a pH range of 5.0-9.6 and survive in food products with pH levels outside these parameters.⁽¹¹⁾

Listeria Enrichment Broth is based on the formula developed by Lovett, et al. in which Tryptic Soy Broth was supplemented with yeast extract for optimum growth of *Listeria* spp.⁽¹²⁾ Modified Listeria Enrichment Broth is an adjusted version of Listeria Enrichment Broth in which the acriflavine content has been reduced from 15.0mg to 10.0mg per liter. This modification reflects the formula specified by the FDA and the International Dairy Federation for isolation of *L. monocytogenes* from milk and milk products.^(5,6)

Modified Listeria Enrichment Broth contains pancreatic digest of casein, papaic digest of soybean meal, and yeast extract as nitrogen and vitamin sources. Dextrose provides an energy source. Sodium chloride maintains the osmotic balance of the medium. Dipotassium phosphate is a buffering agent. Nalidixic acid is incorporated to inhibit growth of gram-negative organisms, while acriflavine is added to suppress growth of gram-positive bacteria. The antifungal cycloheximide is used to inhibit saprophytic fungi.

Identification of *Listeria* spp. is based on successful isolation of the organism, biochemical characterization and serological confirmation.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	17.0gm
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Yeast Extract	6.0gm
Sodium Chloride	5.0gm
Papaic Digest of Soybean Meal	3.0gm
Dextrose	2.5gm
Dipotassium Phosphate	2.5gm
Cycloheximide	0.05gm
Nalidixic Acid	0.04gm
Acriflavine	0.01gm

Final pH 7.3 +/- 0.2 at 25°C.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Consult listed references for information regarding sample preparation and processing.^(1,2,5-12)

For dairy samples:

1. Add 25ml of liquid or 25.0gm of solid test material to 225ml of Modified Listeria Enrichment Broth (Cat. no. U167)

and mix or blend thoroughly.

2. Incubate for 48 hours at 30°C.

3. At 48 hours, streak the Modified Listeria Enrichment Broth culture onto plates of Oxford Medium (Cat. no. G46) or PALCAM Medium (Cat. no. G149).

4. Incubate the agar plates at 37°C. for 48 +/- 2.0 hours.

INTERPRETATION OF RESULTS

Examine plates for typical *Listeria* spp. colonies.

Listeria monocytogenes will show growth with black colonies upon Oxford Medium. *L. monocytogenes* will also display growth with gray-green colonies and black precipitate on PALCAM Medium.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Since the nutritional requirements of organisms vary, some strains of *Listeria* spp. may be encountered that fail to grow or grow poorly on this medium.

Modified Listeria Enrichment Broth is a partially selective medium. Growth of some contaminating strains will be markedly but not totally inhibited.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Listeria monocytogenes</i> ATCC® 7644	A	24-48hr	35°C	Aerobic	Growth
<i>Escherichia coli</i> ATCC® 25922	B	24-48hr	35°C	Aerobic	Partial to complete inhibition
<i>Staphylococcus aureus</i> ATCC® 25923	B	24-48hr	35°C	Aerobic	Partial to complete inhibition

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Modified Listeria Enrichment Broth should appear clear with slight opalescence, and light amber in color.

REFERENCES

1. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
2. Isenberg, H.D. *Clinical Microbiology Procedures Handbook* , Vol. I, II & III. American Society for Microbiology, Washington, D.C.
3. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
4. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.
5. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. AOAC, Arlington, VA.
www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
6. International Dairy Federation. 1990. Milk and milk products - detection of *Listeria monocytogenes* . IDF Provisional International Standard No. 143. International Dairy Federation, Brussels.
7. Murray, E.G, et al. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Listeria monocytogenes* . *J. Path. Bact.* ; 19:407-439.
8. Monk, J.D, et al. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high-fat frozen and refrigerated ground beef. *J. Food Prot.* ; 57:769-772.
9. Wehr, H.M. 1987. *Listeria monocytogenes* - a current dilemma Special Report. *J. Assoc. Anal . Chem.* ; 80:769-7762.
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11. Kramer, P.A., et al. 1969. Media selective for *Listeria monocytogenes* . *J. Appl. Bacteriology* ; 32:381-394.
12. Lovett, J.D., et al. 1987. *Listeria monocytogenes* . *J. Food Prot.* ; 50:188-192.

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