IFU

Instructions for Use

CRITERION™ LEB, SELECTIVE BASE

Cat. no. C9300	CRITERION™ LEB, Selective Base	175.2g
Cat. no. C9301	CRITERION™ LEB, Selective Base	500g
Cat. no. C9302	CRITERION™ LEB, Selective Base	2kg
Cat. no. C9303	CRITERION™ LEB, Selective Base	10kg

INTENDED USE

Hardy Diagnostics CRITERION™ LEB (Listeria Enrichment Broth), Selective Base is recommended for the selective enrichment of *Listeria* spp. from foods or environmental samples. If used in conjunction with rapid pathogen detection systems designed to detect *Listeria* spp. in food or food environment samples, the medium will facilitate the rapid detection of *Listeria* spp.

Dehydrated culture media is a raw material not intended for use in the diagnosis of human disease. For implementation, this product requires additional processing and supplementation of ingredients before use.

SUMMARY

Listeria spp. are microaerophilic, gram-positive regular, short motile rods or coccobacilli that are asporogenous, non-encapsulated, and non-branching. Motility is best observed at 20-25ºC. *Listeria monocytogenes* is a pathogenic organism for humans and a large number of animal species. The members of the population most at risk are neonates, the elderly and those compromised by pregnancy or an underlying illness such as malignancy, alcoholism or some condition which requires immunosuppressive procedures. Intrauterine infection of the fetus results in death, or an acutely ill infant with a septic disseminated form of listeriosis. Papular lesions of the skin may be found in listeriosis of the newborn. A similar cutaneous form has been reported in veterinarians working with infected animals.[1,2]

A common vehicle for *Listeria monocytogenes* is pasteurized milk; since the induction of the Pasteurized Milk Ordinance in 1924, there have been fewer reported cases of milk contaminants other than *Listeria* spp. In Massachusetts in 1983, pasteurized milk spread *Listeria monocytogenes* to forty-nine people, 14 of which died of septicemia. Another incidence in California in 1985, was due to contamination of a soft Mexican cheese which caused 85 deaths of 300 infected patients. This led to a re-evaluation of pasteurization and aging techniques; however, the ability of *L. monocytogenes* to grow between 4 and 10ºC and over a wide pH range (4.4 to 9.6) further complicates the issue. The most effective containment still involves post-pasteurization pathogen detection.[3]

Other types of food that have been found to contain *Listeria* species as a contaminant are raw milk, raw vegetables, fish, poultry, and both fresh and processed meats. Ice cream has also served as a vehicle of transmission and in 1994 shrimp from a party in New York City infected ten people including two pregnant women. The CDC recommends, for immunocompromised, pregnant or elderly individuals, that foods to avoid are: soft cheeses, cold cuts and salami. There are also some reports of nosocomial infections of *Listeria monocytogenes* usually among infants or immunosuppressed adults.[1]

Listeria monocytogenes is ubiquitous in nature and has been isolated from soil, mud, sewage, decaying vegetation, silage, feces, and river water. Many animal species are vulnerable to infection by *Listeria* species and some lactating mammals can function as carriers (with no visible symptoms) while still excreting the organisms in their milk. Sheep, cattle and goats have also been found to shed *Listeria monocytogenes* in their feces. Listeriosis was caused by a meat product (hot dogs) in 1999 in the United States when 101 infections caused 21 deaths. Other contaminated foods include: coleslaw, pate, jellied pork tongue, cooked chicken and smoked mussels.[4]

Hardy Diagnostics CRITERION™ LEB (Listeria Enrichment Broth), Selective Base contains peptone, yeast extract, beef extract, MOPS (morpholinopropanesulfonic acid), pyruvic acid, and growth enhancers which contain nitrogen, amino acids, essential nutrients, and vitamins to promote microbial growth. Dextrose is added as an energy source. Magnesium sulphate and sodium chloride are salts useful for cell culture and osmotic balance. Lithium chloride, selective agents, and ceftazidime hydrate (not included, but required for preparation) help to prevent the growth of unwanted organisms present in the sample. When prepared, the medium may be particularly useful for pathogen detection in foods or environmental samples when using rapid automated pathogen detection systems designed for that purpose.

FORMULA*

Gram weight per liter:	87.6g/L
Dextrose	5.31g
Peptone	19.78g
Yeast Extract	6.76g
Beef Extract	0.96g
MOPS	22.64g
Pyruvic Acid	10.0g
Lithium Chloride	6.5g
Magnesium Sulphate	4.94g
Sodium Chloride	5.86g
Growth Enhancers/Selective Agents	4.85g

Final pH 7.3 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) that contain dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic and will clump when exposed to moisture and air. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. Dehydrated culture media should be discarded if clumped, if the media is not free-flowing or if the color has changed from its original yellow color with dark specks. Do not remove the container desiccant, if applicable

Store the prepared culture media at 2-8ºC in the dark.

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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Combine 87.6g of the dehydrated culture media in one liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat medium to 90ºC while stirring to completely dissolve, then reduce the temperature to 35-40ºC.

3. Weigh out 35mg of ceftazidime hydrate powder (adjusted for potency) and combine with the basal medium. Mix thoroughly.

4. Sterilize the medium using a sterile 0.2µm filter (e.g. sterile disposable Nalgene™ Rapid-Flow™ PES membrane) into a sterile flask or suitable sterile containers for use.

PROCEDURE

Sample Collection: Consult reference methods for information on sample collection. Samples should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the sample should be inoculated onto an appropriate transport medium and refrigerated until inoculation.

Method of Use: Allow the medium to warm to room temperature prior to inoculation. Consult reference methods for information concerning inoculation procedures. Rapid automated pathogen detection systems can be validated for use with this medium to detect *Listeria* spp. in positive cultures. Consult the appropriate manufacturer's technical information for guidance.

INTERPRETATION OF RESULTS

Growth in the form of turbidity (i.e. cloudiness) is indicative of a positive result.

Consult the manufacturer's technical information when using rapid pathogen detection systems to detect the presence of *Listeria* spp.

For traditional identificaiton, positive samples should be subcultured to the appropriate solid medium such as Modified Oxford Medium (Cat. no. G46), HardyCHROM Listeria (Cat. no. G317), or Compact Dry™ Listeria (Cat. no. LS100) to obtain isolated colonies. Well isolated colonies can be further tested using biochemical methods such as Microgen™ Listeria ID (Cat. no. MID67) or latex agglutination (Cat. no. F48) to confirm the presence of *Listeria* in the sample and

to identify to the species level. Consult reference methods for appropriate biochemical identification tests and for interpretation of results.

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Rare, fastidious microorganisms may not grow in selective media formulations.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, incubators, tubes, bottles, ceftazidime hydrate, sterile disposable 0.2µm Nalgene™ Rapid-Flow™ PES filter, petri dishes, prepared culture media (Cat. nos. G46, G317, and LS100) and ready-to-use biochemical tests (Cat. nos. MID67 and F48), etc., 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	
Listeria monocytogenes ATCC® 7644	A	24-30hr	35°C	Aerobic	Growth; turbidity
Enterococcus faecalis ATCC® 29212	A	48hr	35°C	Aerobic	Partial to complete inhibition
Staphylococcus aureus ATCC® 6538	B	48hr	35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ LEB (Listeria Enrichment Broth), Selective Base powder should appear homogeneous, free-flowing,

and yellow in color with some dark specks. The prepared medium should appear clear and amber to dark amber in color.

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.

2. Association of Official Analytical Chemists. *Official Methods of Analysis*, AOAC, Washington, D.C.

3. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.

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*.Arlington, VA
http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

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Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207B [D]