

DEMI-FRASER BROTH BASE (7656)

Intended Use

Demi-Fraser Broth Base is used with ferric ammonium citrate for the selective enrichment of *Listeria* species.

Product Summary and Explanation

Listeria monocytogenes, described in 1926 by Murray, Webb, and Swann, is a widespread problem in public health and food industries.¹ This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and susceptible pregnant women.² Epidemiological evidence from outbreaks of listeriosis indicate the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission include turkey frankfurters, coleslaw, pasteurized milk, Mexican style cheese and pate'.⁴ *Listeria* species are ubiquitous in nature, present in a wide range of unprocessed foods and in soil, sewage, and river waste.⁵

Demi-Fraser Broth Base is a modification of Fraser Broth Base, developed by Fraser and Sperber,⁶ and used for the rapid detection of *Listeria* from food⁷ and environmental samples. In Demi-Fraser Broth Base, the nalidixic acid and acriflavine concentration have been reduced in accordance with AFNOR guidelines.⁸ *Listeria* species grow over a pH range of 5.0 - 9.6, and can survive in food products with pH levels outside these parameters.⁹ Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization, and serological confirmation.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, Beef Extract, and Yeast Extract provide nitrogen, vitamins, and minerals in Demi-Fraser Broth Base. The Phosphates are the buffering agents. Sodium Chloride maintains osmotic balance. Differentiation is aided by including Ferric Ammonium Citrate in the final medium. Since all *Listeria* species hydrolyze esculin, the addition of ferric ions to the medium will detect the reaction. A blackening of the medium by cultures containing esculin hydrolyzing bacteria is the result of formation of 6,7-dihydroxycoumarin that reacts with ferric ions.⁶ Selectivity is provided by the presence of Lithium Chloride, Nalidixic Acid, and Acriflavin in the formula. The high salt tolerance of *Listeria* is used to inhibit growth of enterococci.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Beef Extract	5 g
Yeast Extract.....	5 g
Sodium Chloride	20 g
Disodium Phosphate.....	9.6 g
Monopotassium Phosphate	1.35 g
Esculin	1 g
Acriflavin	0.012 g
Nalidixic Acid.....	0.010 g
Lithium Chloride.....	3 g

Final pH: 7.2 ± 0.2 at 25°C

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

Supplement / 10 mL

5% Ferric Ammonium Citrate, 10 mL
filtered sterilized aqueous solution /L

Precautions

1. For Laboratory Use.
2. TOXIC. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 55 g of the medium in one liter of purified water.
2. Heat gently in a 50°C waterbath to completely dissolve the medium. DO NOT OVERHEAT.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Aseptically add 10 mL of a filtered sterilized 5% aqueous solution of ferric ammonium citrate.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is golden yellow with an amber opalescent top and clear to slightly hazy.

Expected Cultural Response: Cultural response in Demi-Fraser Broth Base at 35°C after 18 - 48 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	growth w / blackening
<i>Listeria monocytogenes</i> ATCC® 15313	growth w / blackening
<i>Staphylococcus aureus</i> ATCC® 25923	suppressed at @ 24 hours; recovery at 48 hours

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

To isolate *Listeria monocytogenes* and other *Listeria* spp., refer to appropriate references.^{7,8,9,10}

Results

Listeria is presumptively indicated by the blackening of Demi-Fraser Broth Base after 18 - 48 hours incubation at 35°C. For further identification and confirmation of *Listeria* species, consult appropriate references.^{7,8,9,10} Rapid slide and macroscopic tube tests can be used for definitive serological identification.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

Demi-Fraser Broth Base	Code No.	7656A	500 g
		7656B	2 kg
		7656C	10 kg

References

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3. Bremer, P. J., and C. M. Osborne. 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58**:604-608.
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5. Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
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9. Vanderzant, C., and D. F. Splittstoesser (eds). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
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Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.