



FRASER BROTH (7626)

Intended Use

Fraser Broth is used with Acriflavin, Nalidixic Acid, and Ferric Ammonium Citrate for the selective enrichment of *Listeria* spp.

Product Summary and Explanation

Listeria monocytogenes, described first in 1926 by Murray, Webb and Swann, is an extensive problem in public health and food industries.¹ This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women.² Epidemiological evidence from outbreaks of listeriosis indicated 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

Fraser Broth is based on the formulation of Fraser and Sperber.⁵ This medium is used in rapid detection of *Listeria* from food⁶ and environmental samples. *Listeria* spp. grow over a pH range of 5.0 - 9.6, and survive in food products with pH levels outside these parameters.⁷ *Listeria* spp. are microaerophilic, Gram-positive, asporogenous, non-encapsulated, non-branching, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* spp. 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 Fraser Broth. 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. Blackening of the medium by esculin-hydrolyzing bacteria is a result of formation of 6,7-dihydroxycoumarin that reacts with ferric ions.⁵ Selectivity is provided by the presence of Lithium Chloride, Nalidixic Acid and Acriflavin in the formula. The high salt tolerance of *Listeria* spp. 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.....	12 g
Monopotassium Phosphate	1.35 g
Esculin	1 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

Acriflavin, 25 mg
Nalidixic Acid, 20 mg

Supplement / 5 mL (# 7984)

Ferric Ammonium Citrate (250 mg)

Precautions

1. For Laboratory Use.
2. Harmful. Harmful if swallowed, inhaled, or absorbed through the skin. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 57.4 g of the medium in one liter of purified water.
2. Stir without heat to obtain solution, if necessary, warm to 50°C. DO NOT OVERHEAT.
3. Autoclave at 121°C for 15 minutes. Cool broth to room temperature.
4. Aseptically add 10 mL of a filter sterilized solution containing 20 mg Nalidixic Acid and 25 mg Acriflavin. Additionally, add 2 x 5 mL Ferric Ammonium Citrate Supplement (7984).

Quality Control Specifications

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

Prepared Appearance: Prepared medium is amber, clear to slightly hazy with trace to moderate precipitate.

Expected Cultural Response: Cultural response in Fraser Broth incubated at $35 \pm 2^\circ\text{C}$ and examined for growth after 18 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Escherichia coli</i> ATCC® 25922	$\sim 10^3$	Suppressed (@ 18 -24 hours); no blackening
<i>Listeria monocytogenes</i> ATCC® 7644	10 - 300	Growth w / blackening
<i>Listeria monocytogenes</i> ATCC® 15313	10 - 300	Growth w / blackening
<i>Staphylococcus aureus</i> ATCC® 25923	$\sim 10^3$	Suppressed (@ 18 – 24 hours); no blackening

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

Test Procedure

To isolate *Listeria monocytogenes* from processed meats and poultry, the following procedure is recommended by the U.S.D.A.⁶

1. Add 25 g of test material to 225 mL of UVM Modified Listeria Enrichment Broth and mix or blend thoroughly. Incubate for 20 - 24 hours at 30°C .
2. Transfer 0.1 mL of the incubate broth to Fraser Broth. Incubate at 35°C for 26 ± 2 hours.
3. At 24 and 48 hours, streak the Fraser Broth culture to Modified Oxford Agar. Incubate the Modified Oxford plates at 35°C for 24 - 48 hours.

Results

1. Examine agar plates for suspect colonies. For complete identification and confirmation of *Listeria* spp., consult appropriate references.^{6,7,8}
2. Rapid slide and macroscopic tube tests can be used for definitive serological identification.

Storage

Store sealed bottle containing the dehydrated medium at $2 - 30^\circ\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

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.

Limitation of the Procedure

An identification of *Listeria monocytogenes* must be confirmed by biochemical and serological testing.⁸

Packaging

Fraser Broth	Code No.	7626A	500 g
		7626B	2 kg
		7626C	10 kg
Ferric Ammonium Citrate, 5 mL		7984	10 vials/ pkg

References

1. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bact. 29:407-439.
2. Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen refrigerated ground beef. J. Food Prot. 57:969-974.
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.
4. Grau, F. H., and P. B. Vanderlinde. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. 55:4-7.

5. **Fraser, J., and W. Sperber.** 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. J. Food Prot. **51**:762-765.
6. **Lee, W. H., and D. McClain.** 1994. Laboratory Communication No. 57, U.S.D.A., F.S.I.S. Microbiology Division, Bethesda, MD.
7. **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.
8. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products 16th ed., American Public Health Association, Washington D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.