# 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 food[7] 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 / 5mL (PN 7984)

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

## Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful if swallowed, inhaled, or absorbed through the skin. Skin irritation may be severe. Irritating to eyes, skin, and respiratory system. May cause central nervous system effects.

## Directions

1. Dissolve 55 g of the medium in one liter of purified water.

2. Mix thoroughly.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Aseptically add 10 mL of Fraser Broth Supplement.

## Quality Control Specifications

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

**Prepared Appearance:** Prepared medium is golden yellow with an amber opalescent top and clear to slightly hazy with none to light precipitate.

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

| Microorganism | Approx. Inoculum (CFU) | Expected Results | |
|---|---|---|---|
| | | Growth | Reaction |
| *Escherichia coli* ATCC® 25922 | 1000 | Inhibited | --- |
| *Listeria monocytogenes* ATCC® 7644 | 10 - 300 | Growth | Blackening |
| *Listeria monocytogenes* ATCC® 15313 | 10 - 300 | Growth | Blackening |
| *Staphylococcus aureus* ATCC® 25923 | 1000 | Partial to complete inhibition | ---- |

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]

## 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] 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.

## 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.

## Limitation 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** |
| **Fraser Broth Supplement** | | **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. Bacteriol. **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 and 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. **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 monoytogenes*. J. Food Prot. **58:**244-250.

6. **Fraser, J., and W. Sperber.** 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. J. Food Prot. **51:** 762-765.
7. **Lee, W. H., and D. McClain.** 1994. Laboratory Communication No. 57, U.S.D.A., F.S.I.S. Microbiology Division, Bethesda, MD.
8. **L'association francaise de normalisation (AFNOR).** 1993. Food Microbiology-Detection of *Listeria monocytogenes*-Routine Method, V 08-055. AFNOR, Paris, France.
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.

## 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.

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