

Demi-Fraser Broth Base Fraser Broth Supplement

Intended Use

Demi-Fraser Broth Base is used with Fraser Broth Supplement in selectively and differentially enriching *Listeria* from foods.

Summary and Explanation

Fraser Broth Base and Fraser Broth Supplement are based on the Fraser Broth formulation of Fraser and Sperber.¹ The medium is used in the rapid detection of *Listeria* from food and environmental samples. Demi-Fraser Broth Base is a modification of Fraser Broth Base in which the nalidixic acid and acriflavine concentrations have been reduced to 10 mg/L and 12.5 mg/L respectively.²

Principles of the Procedure

Peptone, beef extract and yeast extract provide carbon and nitrogen sources and the cofactors required for good growth of *Listeria*. Sodium phosphate and potassium phosphate buffer the medium. Selectivity is provided by lithium chloride, nalidixic acid and acriflavine. The high sodium chloride concentration of the medium inhibits growth of enterococci.

All *Listeria* species hydrolyze esculin, as evidenced by a blackening of the medium. This blackening results from the formation of 6,7-dihydroxycoumarin, which reacts with ferric ions.¹ Ferric ions are added to the final medium as ferric ammonium citrate in Fraser Broth Supplement.

Formulae

Difco™ Demi-Fraser Broth Base

Approximate Formula* Per Liter	
Tryptose	10.0 g
Beef Extract.....	5.0 g
Yeast Extract	5.0 g
Sodium Chloride	20.0 g
Disodium Phosphate	9.6 g
Monopotassium Phosphate	1.35 g
Esculin	1.0 g
Nalidixic Acid	0.01 g
Acriflavine HCl	12.5 mg
Lithium Chloride	3.0 g

Difco™ Fraser Broth Supplement

Formula Per 10 mL Vial	
Ferric Ammonium Citrate	0.5 g

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

Directions for Preparation from Dehydrated Product

1. Dissolve 55 g of the powder in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
3. Aseptically add 10 mL of Fraser Broth Supplement. Mix well.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Demi-Fraser Broth Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in purified water. Solution is medium amber, clear to slightly opalescent, may have a fine precipitate.
Prepared Appearance:	Medium amber, very slightly to slightly opalescent, may have a fine precipitate.
Reaction of 5.5% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Fraser Broth Supplement

Solution Appearance:	Dark brown solution.
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Cultural Response

Difco™ Demi-Fraser Broth Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY/APPEARANCE
<i>Enterococcus faecalis</i>	29212	10 ³ -2×10 ³	Partial to complete inhibition
<i>Escherichia coli</i>	25922	10 ³ -2×10 ³	Inhibition
<i>Listeria monocytogenes</i>	19114	10 ² -10 ³	Good/blackening of the medium
<i>Listeria monocytogenes</i>	19115	10 ² -10 ³	Good/blackening of the medium
<i>Staphylococcus aureus</i>	25923	10 ³ -2×10 ³	Inhibition



Procedure²

1. Pre-enrich the sample in Demi-Fraser Broth. Incubate for 18-24 hours at $35 \pm 2^\circ\text{C}$. Subculture onto Oxford Medium or PALCAM Medium.
2. Transfer 0.1 mL of the pre-enrichment culture into 10 mL of Fraser Broth and incubate for 48 hours at 37°C . Subculture onto Oxford Medium or PALCAM Medium after 18-24 hours and again after 42-48 hours of incubation.
3. Examine Oxford Medium or PALCAM Medium plates for the appearance of presumptive *Listeria* colonies.
4. Confirm the identity of all presumptive *Listeria* by biochemical and/or serological testing.

Expected Results

The presence of *Listeria* is presumptively indicated by the blackening of Demi-Fraser Broth after incubation for 24-48

hours at 35°C . Confirmation of the presence of *Listeria* is made following subculture onto appropriate media and biochemical/serological identification.

References

1. Fraser and Sperber. 1988. J. Food Prot. 51:762.
2. L'association française de normalisation (AFNOR). 1993. Food microbiology- Detection of *Listeria monocytogenes*-Routine method, V 08-055. AFNOR, Paris, France.

Availability

Difco™ Demi-Fraser Broth Base

Cat. No. 265320 Dehydrated – 500 g
265310 Dehydrated – 10 kg

Difco™ Fraser Broth Supplement

Cat. No. 211742 Tube – 6 x 10 mL*

*Store at $2-8^\circ\text{C}$.