Listeria Fraser Broth II

Selective liquid medium for the detection of *Listeria monocytogenes* and *Listeria* spp, according to ISO 11290-1.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Animal Tissues	5.0
Enzymatic Digest of Casein	5.0
Meat Extract	5.0
Yeast Extract	5.0
Sodium Chloride	20.0
Disodium Phosphate, Anhydrous	9.6*
Potassium Dihydrogen Phosphate	1.35
Aesculin	1.0
Lithium Chloride	3.0
Final pH 7.2 ± 0.2 at 25°C	

*Equivalent to 12.0 g of Disodium Hydrogen Phosphate, Dihydrate.

DESCRIPTION

Listeria Fraser Broth II is a liquid medium used with supplements for the selective enrichment of *L monocytogenes* and *Listeria* spp from food, animal feeding and environmental samples in the area of food production and food handling.

The medium is completed after the addition of Listeria Fraser Supplement (FAC-NA), ref. 81628A, which includes the following:

- Listeria Fraser Supplement. This contains Ferric Ammonium Citrate.
- Fraser Nalidixic – Acriflavine Supplement. This contains Acriflavine Hydrochloride and Nalidixic Acid.

The complete medium complies with the recommendations of the ISO 11290-1.

In the ISO methodology, Half Fraser Broth and Fraser Broth are used for the primary and secondary enrichment, respectively. Half Fraser Broth is a modification of Fraser Broth which contains half of the concentration of nalidixic acid and acriflavine.

Supplement	Ref. 81628A (content per vial)	(content per litre of medium)	
		Fraser Broth	Half Fraser Broth
Listeria Fraser Ferric Ammonium Citrate	0.25 g	0.5 g	0.5 g
Fraser Nalidixic – Acriflavine Nalidixic Acid Acriflavine Hydrochloride	 10.0 mg 12.5 mg	 20.0 mg 25.0 mg	 10.0 mg 12.5 mg

PRINCIPLE

Enzymatic digest of animal tissues, enzymatic digest of casein and meat extract provide nitrogen, vitamins, minerals and amino acids for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Sodium chloride maintains the osmotic balance of the medium and in a so high concentration inhibits enterococci. Potassium and sodium phosphates act as buffer system. Aesculin is hydrolyzed by all *Listeria* species to aesculetin. Lithium chloride is inhibitory for the accompanying flora.

Ferric ions provided by ammonium iron(III) citrate will react with aesculetin producing a blackening of the medium. Acriflavine and nalidixic acid are selective agents.

PREPARATION

Suspend 55 g of powder in 1 liter of deionized or distilled water. Bring to boil and shake until completely dissolved. Sterilize at 121°C for 15 minutes. Cool up to 45-50°C. Aseptically, add rehydrated contents of the supplements as follow:

Primary enrichment (Half Fraser Broth): 2 vials of Listeria Fraser Supplement and 1 vial of Fraser Nalidixic – Acriflavine Supplement.

Secondary enrichment (Fraser Broth): 2 vials of Listeria Fraser Supplement and 2 vials of Fraser Nalidixic – Acriflavine Supplement.

TECHNIQUE

1. Add sample to Half Fraser Broth to prepare a 10-fold dilution (w/v or v/v).
   Incubate at 30 ± 1°C for 25 ± 1 h.
2. Transfer 0.1 ml of the primary enrichment culture into 10 ml of Fraser Broth.
   Incubate at 37 ± 1°C for 24 ± 2 h.
3. Streak from both primary and secondary enrichments onto O.A. Listeria Agar (ref. 10620) to obtain well-separated colonies.
   Incubate at 37 ± 1°C for 24 ± 2 h and for an additional 24 ± 2 h.
4. Use the selective enrichments to inoculate a second selective medium, e.g. Listeria Palcam Agar (ref. 10041), Listeria Oxford Agar (ref. 610167). Refer to the relevant technical sheet for further details.

INTERPRETATION OF RESULTS

A blackening of Half Fraser Broth can develop during the incubation.

Blue-green colonies with or without halo on O.A. Listeria Agar are considered presumptive *Listeria* spp. Typical colonies of *L. monocytogenes* are surrounded by an opaque halo.

For confirmation, subculture onto appropriate non-selective agar, e.g. Blood Agar, Nutrient Agar, TSYEA (ref. 10432). Then, carry out confirmation tests including a positive and negative control.

LIOFILCHEM® S.r.l.
Via Scozia, Zona Ind.le - 64026, Roseto degli Abruzzi (TE) - ITALY
Tel +39 0858930745 Fax +39 0858930330 www.liofilchem.com

STORAGE CONDITIONS

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared medium at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

1. EN ISO 11133:2014+Amd1:2018. Microbiology of food, animal feed and water − Preparation, production, storage and performance testing of culture media.
2. ISO 11290-1:2017.Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. – Part 1: Detection Method.
3. ISO 11290-2:2017.Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. – Part 2: Enumeration Method.
4. Rapporto ISTISAN 96/35. ISSN 1123-3117. Metodi di analisi per il controllo microbiologico degli alimenti.
5. Normalisation Francaise, AFNOR (1993) V08-55.
6. Fraser. J.A and Sperber W.H (1988) J. Food Prot , 51, 762-765.

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PRODUCT SPECIFICATIONS

NAME
Listeria Fraser Broth II

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
610618	500 g	500 g of powder in plastic bottle

pH OF THE MEDIUM
7.2 ± 0.2

USE
Listeria Fraser Broth II is a liquid medium used with supplements for the detection of *L. monocytogenes* and *Listeria* spp in food and environmental samples, according to ISO 11290-1

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: beige
Ready-to-use medium
Appearance: clear
Colour: amber

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Incubation Conditions: 24 ± 2 h / 37 ± 1°C

   Inoculum for productivity: ≤100 CFU

Microorganism		Specification
Listeria monocytogenes serovar 4b + *Escherichia coli* + *Enterococcus faecalis*	WDCM 00021 WDCM 00013 WDCM 00009	Blackening of the medium, >10 colonies on O.A. Listeria Agar

   Inoculum for selectivity: >10^3 CFU

Microorganism		Specification
Escherichia coli	WDCM 00013	Total inhibition on TSA
Enterococcus faecalis	WDCM 00009	<100 colonies on TSA

TSA: Tryptic Soy Agar

TABLE OF SYMBOLS

LOT Batch code	Keep away from Sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult instructions for use	Do not reuse

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