

## LISTERIA HALF-FRASER BROTH BASE

FRLB-OHI-500

- **Principle**

Listeria Half-Fraser Broth Base is a modification of Listeria Fraser Broth Base (FRLB-00I-500) in which the nalidixic acid and acriflavine concentrations have been reduced to 10 mg/L and 12.5 mg/L respectively. The antibiotics are already included in the formula, so it is only necessary to add the Ferric Ammonium Citrate Supplement (FACS-00P-010).

Listeria spp. may be present in small numbers and are often accompanied by considerably larger numbers of other microorganisms; therefore, selective enrichment is necessary. Listeria Half-Fraser broth is used in this selective enrichment and enumeration of Listeria monocytogenes and other Listeria species in all food types, including milk and dairy products, and environmental samples. This formula adheres to ISO 11290.

Enzymatic digest of casein, enzymatic digest of animal tissues and meat extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Potassium phosphates act as a buffer system. All Listeria species hydrolyse esculin, which reacts with ferric ions producing a blackening of the medium. The addition of ferric ammonium citrate improves the growth of Listeria monocytogenes. Lithium chloride inhibits the growth of enterococci that can hydrolyse esculin.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 11290.

- **Composition**

Ingredients	g/L
Enzymatic digest of casein	5.00
Beef extract	5.00
Potassium dihydrogen phosphate	1.35
Yeast extract	5.00
Lithium chloride	3.00
Acriflavine hydrochloride	0.0125
Esculin	1.00
Nalidixic acid	0.01
Sodium chloride	20.00
Enzymatic digest of animal tissues	5.00
Disodium hydrogen phosphate dihydrate	12.00

- **Preparation**

Suspend 28.7 grams of the medium in 500 ml. of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50 °C and aseptically add one vial of Ferric Ammonium Citrate Supplement (FACS-00P-010). Homogenize gently and dispense into sterile containers.

- **Applications and use**

Detection of *Listeria monocytogenes* and *Listeria* spp. according to ISO 11290:

- Primary enrichment: Weigh 25 g (or 25 ml) of the sample and add 225 ml of Listeria ½ Fraser Broth Base (FRLB-OHI-500) with the Ferric Ammonium Citrate Supplement (FACS-00P-010) added. Homogenize and incubate at 30 °C for 25±1 hours.

- Secondary enrichment: Inoculate 0.1 ml of the incubated previous medium (regardless of its colour) in 10 ml of Fraser Broth (FRLB-00I-500). Incubate at 37 °C for 24±2 hours under aerobic conditions.

- Plating out and identification: From the primary enrichment culture, inoculate the selective medium of the laboratory choice, to obtain well separated colonies. From the secondary enrichment culture, repeat the procedure, inoculate the surface of the selective medium. Incubate Listeria Agar according to Ottaviani and Agosti for a total of 48±2 h.

- Confirmation: Select the presumptive colonies and carry out confirmatory tests for *L. monocytogenes* or *Listeria* spp.

- **Quality control**

<b>Solubility</b>	w/o rests
<b>Appearance</b>	Fine powder
<b>Colour of the dehydrated medium</b>	Beige
<b>Colour of the prepared medium</b>	Amber
<b>Final pH (25 °C)</b>	7.2 ± 0.2

- **Microbiological test**

According to ISO 11133:

Incubation conditions: Productivity and Selectivity (30±1 °C / 25±1 h)

Inoculation conditions: Target microorganisms (1000 CFU) / Selectivity (10<sup>4</sup>-10<sup>6</sup> CFU)

<b>Microorganisms</b>	<b>ATCC</b>	<b>Specification</b>	<b>Characteristic reaction</b>
Escherichia coli	25922	Total inhibition on TSA	-
Enterococcus faecalis	29212	<100 colonies on TSA	-

Listeria monocytogenes + Escherichia coli + Enterococcus faecalis	13932 + 25922 + 29212	>10 colonies on Agar Listeria according to Ottaviani and Agosti	Blue green colonies with opaque halo
Listeria monocytogenes + Escherichia coli + Enterococcus faecalis	35152 + 25922 + 29212	>10 colonies on Agar Listeria according to Ottaviani and Agosti	Blue green colonies with opaque halo

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **Bibliography**

Fraser. J.A and Sperber W.H (1988) McClain D. and Lee W.H (1988)

ISO 11290 Horizontal method for the detection and enumeration of Listeria monocytogenes.

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.