

Listeria Enrichment Broth Base ISO

Enrichment medium for the detection and enumeration of *Listeria* in food and environmental samples.

Practical information

Applications	Categories
Selective enrichment	Listeria

Industry: Clinical / Food

Regulations: ISO 11133 / ISO 11290



Principles and uses

Listeria Enrichment Broth Base is an appropriate medium for the selective enrichment of *Listeria* in the two-step method according to ISO 11290, for the preparation of Fraser or Half Fraser Broth by adding the respective supplements.

It is recommended for the detection of *Listeria* spp. in food products and in samples from the environment. All *Listeria* species hydrolyze the aesculin to esculetin, which reacts with iron ions producing a blackening of the medium. Another advantage of this medium is that the addition of ammonium iron (III) citrate improves the growth of *L. monocytogenes*. Lithium chloride included in the medium, along with nalidixic acid and acryflavine from the supplement, inhibit the growth of the accompanying flora, which can hydrolyze the aesculin. The high amount of sodium chloride inhibits the growth of enterococci. 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. Phosphate salts act as a buffer system.

Formula in g/L

Enzymatic digest of casein	5	Esculin	1
Beef extract	5	Sodium chloride	20
Yeast extract	5	Enzymatic digest of animal tissues	5
Potassium hydrogen phosphate	1,35	Lithium chloride	3
Disodium hydrogen phosphate dihydrate	12		

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 the Fraser Listeria Selective Supplement (Cat. 6001) for preparing Fraser Broth or one vial of the Half Fraser Listeria Selective Supplement (Cat. 6002) for preparing Half Fraser Broth. Homogenize gently and dispense into sterile containers.

Instructions for use

» For clinical diagnosis, the type of sample is amniotic fluid.

- Inoculate 0,1 ml of the culture of the Half Fraser Broth incubated (regardless of its color) in 10 ml of Fraser Broth. Incubate at 37 °C for 24±2 hours under aerobic conditions.

» For other uses not covered by the CE marking:

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 Enrichment Broth Base Fraser (Cat. 1120) with the Half Fraser Listeria Selective Supplement (Cat. 6002) added. Homogenize and incubate at 30 °C for 25±1 hours.

- Secondary enrichment: Inoculate 0,1 ml of the previous incubated medium (regardless of its color) in 10 ml of Listeria Enrichment Broth Base Fraser with the Selective Supplement for Listeria Fraser (Cat. 6001) added. Incubate at 37 °C for 24±2 hours under aerobic conditions.
 - Plating out and identification: From the primary enrichment culture, inoculate the Listeria Agar acc. to Ottaviani and Agosti (Cat. 1345) and the other selective medium of the laboratory, to obtain well separated colonies.
- From the secondary enrichment culture, repeat the procedure, inoculate the surface of the Listeria Agar according to Ottaviani and Agosti and the other selective medium.
- For Listeria Agar according to Ottaviani and Agosti incubate for a total of 48±2 h.
- Confirmation: Select presumptive colonies and carry out confirmatory tests for *L. monocytogenes* or *Listeria* spp.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige	Amber	7,2±0,2

Microbiological test

According to ISO 11133:

Half Fraser Broth:

- Incubation conditions: (30±1 °C / 24±2 h).
- Inoculation conditions: Target microorganisms (<100 CFU) / Non-target microorganism (>1000 CFU) / Selectivity (10⁴-10⁶ CFU).

Fraser Broth:

- Incubation conditions: (37±1 °C / 48±2 h).
- Inoculation conditions: Target microorganisms (<100 CFU) / Non-target microorganism (>1000 CFU) / Selectivity (10⁴-10⁶ CFU).

Microorganisms	Specification	Characteristic reaction
<i>Escherichia coli</i> ATCC 25922	Total inhibition (0)	
<i>Enterococcus faecalis</i> ATCC 29212	<100 colonies in TSA	
<i>Listeria monocytogenes</i> ATCC 13932 + <i>Escherichia coli</i> ATCC 25922 + <i>Enterococcus faecalis</i> ATCC 29212	> 10 colonies in Listeria Agar according to Ottaviani and Agosti	Bluish-green colonies with opaque halo
<i>Listeria monocytogenes</i> ATCC 35152 + <i>Escherichia coli</i> ATCC 25922 + <i>Enterococcus faecalis</i> ATCC 29212	> 10 colonies in Listeria Agar according to Ottaviani and Agosti	Bluish-green colonies with opaque halo

Storage

Temp. Min.: 2 °C
Temp. Max.: 25 °C

Bibliography

ISO Normative 11290-1 Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method.

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