

Specification

Sterile selective supplement used for *Listeria* enrichment according to ISO 11290-1:2006.

Presentation

10 Freeze dried vials
Vial
with: 6 ± 0.1 g

Packaging Details

$22 \pm 0.25 \times 55 \pm 0.5$ mm glass vials, tag labelled, White plastic cap - 10 vials per box.

Shelf Life

49 months

Storage

2-25 °C

Composition

Composition (g/vial)

Sodium Nalidixate.....0.0050
Acriflavine.....0.0062
Ammonium Ferric citrate..... 0.2500

Reconstitute the original freeze-dried vial

by adding :

Sterile Distilled Water..... 6 ml

Description /Technique

Description:

Half Fraser Listeria Selective Supplement (Cat. 6002) is added to Listeria Enrichment Broth Base medium (Cat. 1120) to create the Half Fraser Broth.

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 acriflavine 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.

Technique:

Aseptically reconstitute 1 vial with 6 ml of sterile distilled water. Mix gently until complete dissolution. Aseptically add the content of the vial to 500 ml of Listeria Enrichment Broth Base (Cat. 1120) autoclaved and cooled to 50 °C. Mix well and distribute 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 1/2 Fraser Broth incubated (regardless of its color) in 10 ml of Fraser Broth. Incubate at 37 °C for 24±2 hours under aerobic conditions.

- Plating out and identification: From the primary enrichment culture, Listeria Chromogenic Agar Base according to Ottaviani and Agosti (Cat. 1345) and the other selective medium of the laboratory, are inoculated in order to obtain well separated colonies.

From the secondary enrichment culture, the procedure is repeated, inoculating the Listeria Chromogenic Agar Base according to Ottaviani and Agosti and the other selective medium.

For Listeria Chromogenic Agar Base 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.

» For other uses not covered by the CE marking:

Detection and enumeration 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 Chromogenic Agar Base 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 Chromogenic Agar Base 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.

Quality control

Physical/Chemical control

Color : Dark Orange - Brown - Ochre pH: at 25°C

Microbiological control

Prepare tubes - Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity).

Microbiological control according to ISO 11133:2014/A1:2018.

Aerobiosis. Incubation at 30 ± 1 °C during 24 ± 2 h.

Microorganism

Escherichia coli ATCC® 8739 (1)

Enterococcus faecalis ATCC® 19433 (2)

Listeria monocytogenes ATCC® 13932 + (1) + (2)

Listeria monocytogenes ATCC® 35152 + (1) + (2)

Sterility Control

Incubation 24h at 30-35 °C and 72 h at 20-25 °C: NO GROWTH.

Incubation 7 days at 32.5 ± 2 °C and 7 days at 22.5 ± 2 °C: NO GROWTH.

Add 5ml of the sample to 100 ml of TSB and to 100 ml Thioglycollate.

Growth

Inhibited. Confirm in TSA at 37°C±1 reading 24 ± 3h

Partial Inhibition. Confirm in TSA at 37°C±1 reading 24 ± 3h.

> 10 CFU. Blue-green coln. w. opaque halo (Ottaviani Agosti)

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Bibliography

- ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Florida.
- FRASER, J.A. & W.H. SPERBER (1988) Rapid detection of *Listeria* spp. In food and environmental samples by esculin hydrolysis. J. Food Prot. 51:762-765.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 11290-1:2017 Standard. Microbiology of the food chain. Horizontal method for the detection and enumeration of *Listeria monocytogenes* and for *Listeria* spp.- Part 1: Detection Method
- ISO 11290-2:2017 Standard. Microbiology of the food chain. Horizontal method for the detection and enumeration of *Listeria monocytogenes* and for *Listeria* spp.- Part 2: Enumeration Method.
- McCLAIN, D. & W.H. LEE (1988) Development of a USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. J.AOAC 71:660-664.
- VANDERZANT, C & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington. DC.