

Specification

Supplement that enhances the growth of *Listeria spp.* according to ISO 11290.

Presentation

10 Prepared bottles

with: 24 ± 0.3 ml

Packaging Details

1 box with 10 bottles of 60 ml (total capacity). Injectable cap: Plastic screw inner cap + elastomer septum + protective outer cap.

Shelf Life

24 months

Storage

4-12 °C

Composition

Composition (bottle):

Lipase C substrate..... 24.0 ml

Note: Each vial is sufficient to supplement 470 ml of *Listeria* Agar Base according to Ottaviani and Agosti

Description /Technique

Description:

Completed with all its supplements the Agar *Listeria* Ottaviani & Agosti is a selective and differential medium for the detection of *Listeria* species and the presumptive identification of *Listeria monocytogenes*.

The selectivity is achieved by the high concentration of lithium chloride and the mixture of antimicrobics. The differential activity is due to the chromogenic substrate to detect the β -glucosidase enzyme that is present in all *Listeria* species.

The specific identification is obtained by the L- α -phosphatidylinositol, that acts as substrate for a phospholipase C present only in *Listeria monocytogenes* and some strains of *Listeria ivanovii*. The combination of both substrates allows the differentiation *L. monocytogenes*, which grow in produces colonies blue-green in colour and surrounded by an opaque zone, from the other *Listeria* species, which blue-green colonies but without any halo. This differentiation is evident after incubating the plates for 24 ± 2 hours at 37 °C.

Sometimes, especially with highly contaminated samples, it is possible that some colonies, white in colour, are not *Listeria* growth. In this case an enrichment step is recommended prior to plate inoculation.

Observations: Most *Listeria ivanovii* also produce an opaque halo around the colonies after 48 h of incubation. This presumptive evidence must be confirmed by performing the biochemical or serological identification tests (Rhamnose / Xylose sugar fermentation, hemolysis tests, CAMP test, etc.) or any test confirming the species without hesitation.

Technique:

Add 1 bottle enrichment supplement Ottaviani & Agosti (24 ml) and 1 vial selective supplement Ottaviani & Agosti for complete 500 ml medium.

Homogenize by mixing and distribute in Petri dishes. The solidified cool medium appears homogeneously turbid.

There are many standardised methodologies (ISO, FDA-BAM, AOAC, AFNOR, etc.). The technician must follow the protocol validated in his laboratory.

Quality control

Physical/Chemical control

Color : yellow

pH: at 25°C

Microbiological control

Spiral Spreading: Practical range 100 ± 20 CFU. min. 50 CFU (productivity) / 10⁴-10⁶ CFU (selectivity).

Add to *Listeria* medium base

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

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020

Aerobiosis. Incubation at 37 °C ± 1, reading after 44 ± 4h

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

Microorganism

L. monocytogenes ATCC[®] 13932, WDCM 00021

Listeria innocua ATCC[®] 33090, WDCM 00017

Enterococcus faecalis ATCC[®] 29212, WDCM 00087

Escherichia coli ATCC[®] 25922, WDCM 00013

Listeria monocytogenes ATCC[®] 35152

Sterility Control

Incubation 48 hours at 30-35 °C and 48 hours at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

Growth

Good - Blue colonies with white halo

Blue colonies without white halo

Inhibited

Inhibited

Blue-green colonies with opaque halo

Bibliography

- Artault, S., j.L. Bind, Y. Delaval, N. Dureuil, N. Gallart (2000) AFNOR validation of the ALOA method for the detection of *Listeria monocytogenes* in foodstuffs. Coll. Soc. Fran. Microbiol. 19-20 Oct. Paris.
- Bannerman, E.S. & J. Bille (1988) A new selective medium for isolating *Listeria* from heavily contaminated material. Appl. Environm. Microbiol. 54:1:165-167.
- Greenwood, M., C. Willis, P. Dosweell, G. Allen & K. Pathak (2005) Evaluation of chromogenic media for the detection of *Listeria* species in food.
- Hitchins, A.D. & K. Jinneman (1998) *Listeria monocytogenes* in FDA-BAM 8th edition Revision A. Updater January 2003. AOAC Intl. Gathersburg. MD. USA.
- 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
- Jantzen, M.M., J. Navas, M. de Paz, B. Rodriguez, W.P. da Silva & M. Nuñez (2006) Evaluation of ALOA plating medium for its suitability to recover high pressure-injured *Listeria monocytogenes* from ground chicken meat. Letters Appl. Microbiol 43:313-317
- Manafi, M. W. Kneifel & S. Bascomb (1991) Fluorogenic and chromogenic substrates used in bacterial diagnostics. Microbiol Rev. 55:3:335-348
- Ottaviani, F., M. Ottaviani & M. Agosti (1997) Esperienza su un agar selettivo e differenziale per *Listeria monocytogenes*. Industrie Alimentari 36:1-3
- Victor Lachica, R. (1990) Selective plating medium for quantitative recovery of food-borne *Listeria monocytogenes*. Appl. Environm. Microbiol. 56:1:167-169
- Watkins, J. & K.P. Sleath (1981) Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge and river water. J. Appl. Bacteriol. 50:1-9
- UNE-EN ISO 11133 (2014). Microbiología de los alimentos para consumo humano, alimentación animal y agua.-Preparación, producción, conservación y ensayos de rendimiento de los medios de cultivo.