



Reference : 01-719

Scharlau Microbiology - Technical data sheet

Product :
Microinstant® Listeria Agar Base (Ottaviani and Agosti)



Specification

Medium for the isolation of *Listeria spp.* and the presumptive identification of *L. monocytogenes* acc. to ISO 11290-1 and 11290-2 standard.

Formula * in g/L

Meat peptone.....	18.000	Lithium chloride.....	10.000
Tryptone.....	6.000	Disodium phosphate anhydrous.....	2.500
Yeast extract.....	10.000	5-bromo-4-chloro-3-indolyl-	
Sodium pyruvate.....	2.000	β-D-glucopyranoside.....	0.050
Dextrose.....	2.000	Agar.....	13.000
Magnesium glycerophosphate.....	1.000		
Magnesium sulphate.....	0.500	Final pH 7.2 ± 0.2 at 25 °C	
Sodium chloride.....	5.000		

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 35 g of powder in 470 ml of distilled water and bring to the boil with constant stirring. Sterilise by autoclaving at 121 °C for 15 minutes. Cool to 45-50 °C and add 1 bottle of Listeria Enrichment Supplement(Ottaviani & Agosti) (Ref.06-754-024) and 1 vial of Listeria Selective Supplement (Ottaviani & Agosti) (Ref. 06-755LYO1). Homogenize by mixing and distribute in Petri dishes. The solidified cool medium appears homogeneously turbid.

Description

Completed with all its supplements the Agar Listeria O&A 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 that is present only in Listeria monocytogenes and some strains of Listeria ivanovii.

The combination of both substrates allows the differentiation L. monocytogenes that produces colonies blue-green in colour but surrounded by an opaque zone from the other Listeria species that growth with blue-green colonies without any halo. This differentiation is evident after incubate the plates for 24±2 hours at 37 °C.

Sometimes, especially with highly contaminated samples it is possible that can growth some colonies, white in colour, that are not Listeria. In this case it is recommended an enrichment step previous to the 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 (Ramosa / Xylose sugar fermentation, hemolysis tests, CAMP test, etc.) or any test confirming the species without hesitation.

Remarks:

Enrichment Supplement for Agar Listeria O&A (06-754-024):

1 vial sufficient amount for 500ml complete medium

L-α-phosphatidylinositol..... 1,00 g
Sterile Distilled Water..... 24,00 ml

Selective Supplement for Agar Listeria O&A (06-755LYO1):

1 vial sufficient amount for 500ml complete medium

Nalidixic acid..... 10 mg
Ceftazidime..... 10 mg
Cycloheximide..... 25 mg
Polymyxin B sulphate..... 38350 ui

Reconstitute the original freeze-dried vial by adding 1 vial with
Sterile Distilled Water..... 6,00 ml



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Technique

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

Quality control

Incubation temperature: 37 ± 1 °C

Incubation time: 44±4 h

Inoculum: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10⁴ -10⁶ CFU (selectivity)/ ≥ 10³ CFU (specificity). according to ISO 11133:2014/Amd 1:2018 & Adm 2:2020

Microorganism

Growth

Remarks

Escherichia coli ATCC® 25922

Inhibited

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Listeria monocytogenes ATCC® 13932

Productivity > 0.50

green-blue colonies surrounded by opaque halo

Listeria monocytogenes ATCC® 35152

Productivity > 0.50

green-blue colonies surrounded by opaque halo

Listeria innocua ATCC® 33090

Good (specificity)

green-blue colonies without opaque halo

Enterococcus faecalis ATCC® 29212

Inhibited

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References

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- ISO 11133:2014/ Adm 1:2018/ Adm 2:2020/ 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
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).