Listeria Lipase C Supplement

Cat. 6031

Selective supplement for the isolation, enumeration and detection of Listeria monocytogenes.

Practical information

Aplications	Categories
Selective enumeration	Listeria
Detection	Listeria

Industry: Clinical / Food

Regulations: ISO 11290

Principles and uses

Listeria Chromogenic Agar Base acc. to Ottaviani and Agosti (ALOA) is a selective medium for the presumptive isolation and identification of Listeria monocytogenes and Listeria spp. in food and clinical samples. It is used for confirmation after using Listeria Enrichment Broth Base Fraser (Cat. 1120). This medium is also recommended by ISO 11290-1 for the detection and enumeration for Listeria monocytogenes.

Enzymatic digest of animal tissues and enzymatic digest of casein provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium pyruvate is a source of energy for bacterial metabolism and aids in the resuscitation of stressed organisms. Glucose is the fermentable carbohydrate providing carbon and energy. Magnesium glycerophosphate is a buffering compound. Magnesium sulphate provides magnesium ion required for a large variety of enzymatic reactions, including DNA replication. The differential activity of the medium is due to two factors. Lithium chloride in the base medium and supplementary antimicrobial compounds Ceftazidime, Polymyxin, Nalidixic acid and Cycloheximide provide the medium's selectivity. Bacteriological agar is the solidifying agent.

The presence of the chromogenic component X-glucoside, a substrate for the detection of the enzyme ß-glucosidase, is common to all Listeria species giving the colonies their blue colour. Other organisms that possess this enzyme, for example, Enterococci, are inhibited by the selective agents within the medium and by the selective supplement. The differential activity is also obtained by lipase C substrate, upon which the specific enzyme for L. monocytogenes acts. The lipase is responsible for the opaque white halo which surrounds L. monocytogenes.

The combination of both substrates allows us to differentiate the colonies of Listeria monocytogenes from the rest of Listeria spp. since, although all are blue in colour, L. monocytogenes present an opaque white halo surrounding them.

It has been observed that some strains of Listeria ivanovii, mostly pathogenic to animals although some have caused infections in humans, also possess lipase activity.

Formula per vial

Lipase C Substrate (ml)	10

Preparation

Aseptically add 1 vial of Listeria Lipase C Supplement (Cat. 6031) and 1 vial of Listeria Chromogenic Selective Supplement (ISO 11290-1) (Cat. 6040), previously reconstituted with 6 ml of warm sterile distilled water, to 500 ml of Listeria Chromogenic Agar Base (ISO 11290-1) Acc. to Ottaviani and Agosti (ALOA) (Cat. 1345) autoclaved and cooled to 50-70 ºC. Mix well and distribute into sterile containers. We recommend to add the supplement to the base medium at a temperature higher than normal, around 70 ºC and shake it strongly.

Instructions for use

Detection method:
- Weigh 25 g (or 25 ml) of the sample and add 225 ml of Half Fraser Broth (Cat.1183). Homogenize and incubate at 30 °C for 25±1 hours.
- Inoculate 0,1 ml of incubated Half Fraser Broth culture (regardless of its colour) into 10 ml of Fraser Broth (Cat.1182).
Incubate at 37 ºC for 24±2 hours in aerobic conditions.
- From the primary enrichment culture inoculate the surface of the Agar Listeria according to Ottaviani and Agosti and the other selective medium at the choice of the laboratory, to obtain well-separated colonies.
From the secondary enrichment culture, repeat the procedure, inoculate the surface of the Agar Listeria according to Ottaviani and Agosti and the other

selective medium.
For Agar Listeria according to Ottaviani and Agosti incubate for a total of 48±2 h.
- Select the presumptive colonies and carry out the confirmation tests for L. monocytogenes or Listeria spp.

Enumeration method:
- Prepare an initial suspension 1:10 of sample and Buffered Peptone Water for analysis. Listeria 1/2 Fraser Broth (Cat. 1183) can be used as a diluent if the detection and enumeration procedures are carried out simultaneously.
- Inoculate 0,1 ml on the surface of Listeria Chromogenic Agar according to Ottaviani and Agosti.
- Incubate at 37 ºC for 24±2 h. Incubate for an additional 24 hours in case no microbial growth is detected.
- Select the presumptive colonies and carry out confirmation tests for L. monocytogenes or Listeria spp.
- Calculate from the confirmed colonies the number of L. monocytogenes or Listeria spp colonies.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
Cloudy	Liquid	N/A	Opaque light brown	N/A

Microbiological test

According to ISO 11133:
Incubation conditions: Productivity, Selectivity and Specificity (37±1 ºC / 48±4 h).
Inoculation conditions: Productivity qualitative (10^3-10^4 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU).
Reference media: TSA

Microrganisms	Specification	Characteristic reaction
Listeria monocytogenes 4b ATCC 13932	Good growth (2) >50%	Blue green colonies with opaque halo
Enterococcus faecalis ATCC 29212	Total inhibition (0)	
Listeria innocua ATCC 33090		Blue green colonies without opaque halo
Listeria monocytogenes 1/2a ATCC 35152	Good growth (2) >50%	Blue green colonies with opaque halo
Escherichia coli ATCC 8739	Total inhibition (0)	

Storage

Temp. Min.:2 ºC
Temp. Max.:8 ºC

Bibliography

Ottaviani, F., Ottaviani, M. and Agosti, M (1987) Quimper Froid Symposium Proceedings, P6 A.D.R.I.A Quimper (F) 16-18 June ISO 11290-1:2004 Horizontal method for the detection and enumeration of Listeria monocytogenes Part 1: Detection Method.