

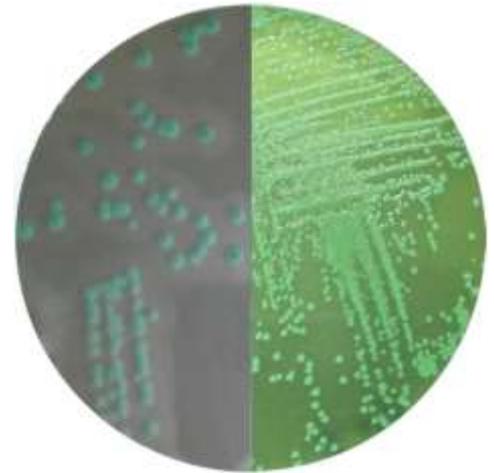
## LISTERIA CHROMOGENIC AGAR BASE (ISO 11290-1) Acc. to OTTAVIANI and AGOSTI (ALOA)

**CAT N°: 1345** Selective medium for the detection and enumeration of *Listeria monocytogenes*

### FORMULA IN g/l

Enzymatic Digest of Animal Tissue	18.00	Glucose	2.00
Lithium Chloride	10.00	Sodium Pyruvate	2.00
Yeast Extract	10.00	Magnesium Glycerophosphate	1.00
Enzymatic Digest of Casein	6.00	Magnesium Sulfate	0.50
Sodium Chloride	5.00	5 Bromo- 4 chloro-3-indolyl β-D glucopyranoside	0.05
Disodium Hydrogen Phosphate	2.50	Bacteriological Agar	13.50

**Final pH 7.2 ± 0.2 at 25°C**



*Listeria monocytogenes*  
ATCC 19111

*Listeria innocua*  
ATCC 33090

### PREPARATION

Suspend 35.275 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. To prepare more quantity of 500 ml, it is recommended to sterilize at 115 ° C for 10 minutes. Cool to 70°C to aseptically add one vial of Listeria Lipase C Supplement (Cat. 6031) and shake it strongly. Then, cool to 50°C and aseptically add one vial of Listeria Chromogenic Selective Supplement (Cat. 6040), previously reconstituted in 6 ml of warm sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8-15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

### LISTERIA LIPASE C SUPPLEMENT (Cat. 6031)

(1 vial for 500 ml of the medium)

We recommend adding the supplement at a temperature higher than normal, around 70 ° C and shaking it strongly.

Lipase C Substrate .....10 ml

### LISTERIA CHROMOGENIC SELECTIVE SUPPLEMENT (Cat. 6040)

(1 vial for 500 ml of the medium)

Cycloheximide .....25 mg

Ceftazidime .....10 mg

Nalidixic acid .....10 mg

Polymyxin B .....38350 IU

Caution: Listeria Chromogenic Selective Supplement contains Cycloheximide and it is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye face protection.

### USES

LISTERIA CHROMOGENIC AGAR BASE 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*.

Meat peptone and Tryptone 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 resuscitation of stressed organisms. Glucose is the fermentable carbohydrate providing carbon and energy. Magnesium glycerophosphate is a buffering compound. Magnesium sulphate is a magnesium ion required in a large variation 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  $\beta$ -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.

## MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with Listeria Chromogenic Selective Supplement (Cat. 6040) & the Listeria Chromogenic Lipase C Supplement (Cat. 6031) added, from type cultures after incubation at a temperature of 37 °C during 24±3 hours. Incubate the negative strains for an extra 24± 3 hours.

Microorganisms	Growth	Colony Color	Halo
<i>Listeria monocytogenes</i> ATCC 19111	Good	Blue	+
<i>Escherichia coli</i> ATCC 25922	Inhibited		-
<i>Listeria innocua</i> ATCC 33090	Good	Blue	-
<i>Enterococcus faecalis</i> ATCC 19433	Inhibited		-

**According to ISO 11133** (44 ±4) h/37±1 °C (Productivity Selectivity and Specificity)

Microorganisms	Inoculum (cfu)	Productivity Qualitative	Selectivity Qualitative	Specificity Qualitative	Reference media	Productivity Quantitative	Characteristic reaction
<i>Listeria monocytogenes</i> ATCC 13932	10 <sup>2</sup>	Good growth (2)			TSA	P <sub>R</sub> ≥0.5	Blue green colonies with opaque halo
<i>Escherichia coli</i> ATCC 8739	10 <sup>4</sup> -10 <sup>6</sup>		Total inhibition (0)				
<i>Enterococcus faecalis</i> ATCC 29212	10 <sup>4</sup> -10 <sup>6</sup>		Total inhibition (0)				
<i>Listeria innocua</i> ATCC 33090	10 <sup>3</sup> -10 <sup>4</sup>			Blue green colonies without opaque halo			

## 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.



## STORAGE

Once opened keep powdered medium closed to avoid hydration.

