

OXFORD LISTERIA SELECTIVE SUPPLEMENT

OLSS-00I-010

- **Principle**

Oxford Listeria Selective Supplement is a freeze-dried selective supplement intended for use with Listeria Agar Base Oxford (LOMB-001) for the detection and isolation of *Listeria monocytogenes* and other *Listeria* spp., from food products and clinical samples. It can be used for direct sample inoculation or as part of a confirmation procedure following a previous enrichment step.

The differential principle of the medium is based on the ability of *Listeria* species to hydrolyse esculin to esculetin. Esculetin subsequently reacts with iron ions present in the medium, producing characteristic black colonies and a blackening of the surrounding medium. This reaction facilitates the presumptive identification of *Listeria* colonies.

The basal medium provides the nutrients required for growth through components such as peptones and maize starch, while Ferric ammonium citrate contributes to the development of the characteristic esculin reaction and supports the growth of *L. monocytogenes*.

Selectivity is achieved through the combined action of lithium chloride and the antimicrobial agents contained in the supplement, which inhibit Gram-negative bacteria and a large proportion of Gram-positive accompanying flora. Cycloheximide additionally suppresses yeast growth, helping to improve the selective recovery of *Listeria* spp.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 11290.

- **Composition**

Ingredients	g/vial
Cycloheximide	0,2000
Colistin sulphate	0,0100
Acriflavine	0,0025
Cefotetan	0,0010
Phosphomycin sodium salt	0,0050

- **Preparation**

Under aseptic conditions, reconstitute 1 vial with 9 ml of sterile solvent consisting of a 1:1 mixture of water and ethanol. Mix gently until the contents are completely dissolved.

Aseptically add the reconstituted supplement to 500 ml of Listeria Agar Base Oxford (LOMB-001), previously sterilized and cooled to approximately 50 °C. Mix thoroughly to ensure homogeneous distribution of the supplement and dispense the complete medium into sterile containers.

- **Application and use**

The supplemented medium is intended for the selective isolation and presumptive detection of *Listeria monocytogenes* and other *Listeria* spp., particularly in food samples. It may be used for direct sample inoculation or as a selective plating medium following an appropriate enrichment procedure.

For clinical diagnosis, the type of sample is amniotic fluid.

- Inoculate on the surface making parallel striae with the handle or hyssop.
- Incubate in aerobic conditions at 37 °C for 48 hours.
- Reading and interpretation of the results.

For the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. according to ISO 11290:

- Weigh 25 g (or 25 ml) of the sample and add 225 ml of 1/2 Fraser Broth (FRLB-0HI). Homogenize and incubate at 30 °C for 25 ± 1 hours.
- Inoculate 0,1 ml of culture of the 1/2 Fraser Broth incubated (independently of the color) in 10 ml of Fraser Broth (FRLB-00I). Incubate at 37 °C for 24 ± 2 hours under aerobic conditions.
- The primary enrichment culture is inoculated on the Listeria Agar surface according to Ottaviani and Agosti (ALOA-00I) and in another selective medium of the laboratory (Oxford), to obtain well separated colonies.
- From the secondary enrichment culture, repeat the procedure, inoculate the surface of the Listeria Agar according to Ottaviani and Agosti (ALOA-00I) and the Oxford Agar. Incubate for a total of 48 ± 2 h.
- Select presumptive colonies and carry out confirmatory tests for *L. monocytogenes* or *Listeria* spp.

Although typical colonies of *L. monocytogenes* are almost always visible after 24 hours of incubation, the incubation should be continued for a further 24 hours to obtain strains of slower growth.

- **Quality control**

Colour	Yellow-orangey
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- **Microbiological control**

Distribute the complete medium, previously cooled to approximately 50 °C, in plates and allow it to solidify.

For performance testing, inoculate the medium using approximately 100 ± 20 CFU, with a minimum of 50 CFU for productivity testing, and 10⁴–10⁶ CFU for selectivity testing.

Incubate under aerobic conditions at 35 ± 2 °C and evaluate the results after 24–48 hours.

Microorganism	ATCC	WDCM	Growth	Esculin Reaction
<i>Escherichia coli</i>	25922	00013	Inhibited	-
<i>L. monocytogenes</i>	13932	00021	Good	Positive
<i>Listeria monocytogenes</i>	35152	-	Good	Positive
<i>Enterococcus faecalis</i>	29212	00087	Inhibited	-

- **Storage and shelf life**

Shelf life: 49 months. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

- **Sterility control**

Sterility is verified by inoculating 5 ml of the sample into 100 ml of TSB and 100 ml of Thioglycollate. The inoculated media is incubated for 48 hours at 30–35 °C and 48 hours at 20–25 °C. No microbial growth shall be observed after the specified incubation periods.

- **Bibliography**

ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Florida.

CURTIS, G.D, R.G. MITCHELL, A.F. KING & E.J. GRIFFIN (1989) A selective differential medium for the isolation of *Listeria monocytogenes*. Letters Appl. Microbiol. 8:95-98.

ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

ISO 11290 standard (1996) Microbiology of food and animal feeding stuff. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1 - Detection method. Part 2. Enumeration method.

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

VANDERZANT, C. & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington DC.

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

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.