

PALCAM LISTERIA SELECTIVE SUPPLEMENT ISO

PLSS-00I-010

- **Principle**

PALCAM Listeria Selective Supplement ISO is a sterile selective supplement designed to be added to Palcam Agar Base (APAL-001) to obtain a complete selective medium for the detection and isolation of *Listeria monocytogenes* from food samples.

The selectivity of Palcam medium is achieved through the combined action of selective agents such as acriflavine, polymyxin B and ceftazidime, together with lithium chloride present in the base medium. These compounds inhibit the growth of most accompanying Gram-negative bacteria and many Gram-positive microorganisms, facilitating the recovery and differentiation of *Listeria* spp.

The differential properties of the medium are based on esculin hydrolysis. *Listeria* spp. hydrolyses esculin to esculetin, which reacts with ferric ammonium citrate to produce a dark precipitate, resulting in characteristic green-grey colonies with beige halos and darkening of the surrounding medium. Enterococci and staphylococci that may grow can generally be differentiated by their ability to utilise mannitol, producing yellow colonies and halos against the cherry-red background of the medium.

However, when a high number of *Listeria* colonies is present, extensive esculin hydrolysis may cause generalized darkening of the medium, which can interfere with colony differentiation. In such cases, inoculation of a more diluted sample is recommended to facilitate colony isolation and interpretation.

- **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.

The analytical methodology and microbiological control comply with applicable international standards, such as to ISO 11133:2014/A1:2018; A2:2020.

- **Composition**

Ingredients	g/vial
Polymyxin B	0,0050
Acriflavine	0,0025
Ceftazidimine	0,0100

- **Preparation**

Collect and prepare the samples, including any required dilutions and test volumes, in accordance with the applicable specifications, official standards, regulatory requirements, and expected analytical results.

Under aseptic conditions, reconstitute the vial with 6 ml of sterile distilled water and mix until completely dissolved. Add the reconstituted supplement to 500 ml of sterilized Palcam Agar Base (APAL-001), previously cooled to approximately 50 °C, and mix it to ensure homogeneous distribution. Do not overheat the medium after supplementation.

Pour the complete medium into sterile Petri dishes and allow it to solidify on a flat surface. Once solidified, spread the plates using an appropriate streaking or spiral method. Incubate under aerobic conditions at 37 ± 1 °C for 44 ± 4 hours. Depending on the sample type or the requirements of the applicable method, alternative incubation temperatures or longer incubation periods may be necessary.

After incubation, examine and enumerate the colonies growing on the agar surface, paying particular attention to any blackening of the medium caused by esculin hydrolysis, a characteristic reaction associated with *Listeria* strains. Any presumptive *Listeria* spp. must subsequently be confirmed by appropriate microbiological and biochemical tests.

- **Application and use**

Recommended to use for the isolation of *Listeria* spp.

- **Quality control**

Colour	Orange
--------	--------

- **Microbiological control**

Isolation by streaking with an inoculating loop.

Incubate under aerobic conditions at 37 ± 1 °C and evaluate the results after 44 ± 4 hours

Microorganism	ATCC	WDCM	Growth	Esculin Reaction
<i>L. monocytogenes</i>	13932	00021	Good	Positive
<i>Escherichia coli</i>	25922	00013	Inhibited	-
<i>Enterococcus faecalis</i>	29212	00087	Inhibited	-
<i>L. monocytogenes</i>	7644	-	Good	Positive

- **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 are 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. Check at 7 days after incubation in same conditions.

- **Bibliography**

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

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

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

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

Van NETTEN, P., J. PERALES, A. van de MOOSDUCK, G.D.W. CURTIS & D.A.A. MOSSEL (1989) Liquid and solid selective differential media for the detection and enumeration of *Listeria monocytogenes*. Int. J. Food Microbiol. 8:299-316

- **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.