Reference: DSHB3052 **A.B.E. - Technical Data Sheet**

Product: PALCAM Agar Selective Supplement for *Listeria*

Specification

A sterile selective supplement used for the isolation of *Listerias spp.*

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials Vial with: 3 ± 0.1 g	23x60 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-25 ºC

Composition

Compositon (g/vial)

Polymyxin B	0.0050
Acriflavine	0.0025
Ceftazidime	0.0100

NOTE : Each vial is sufficient to supplement 500 ml of Listeria Palcam Agar.

Reconstitute the original freeze-dried vial by adding
Sterile Distilled Water.....6 ml

Description /Technique

Description:
Listeria PALCAM selective supplement is added to PALCAM Medium base in order to obtain a complete selective medium used for the detection and the isolation of *Listeria monocytogenes* from foods.
Palcam Agar is based on the formulation described initially by van Netten *et al.* which has a high selectivity and produces good colonial differentiation. Selectivity is achieved by the inclusion of lithium chloride, acriflavine, polymyxin B and ceftazidime, since they inhibit the growth of almost all the Gram negative and most of the Gram positive companion bacteria.
Listeria hydrolyze esculin to esculetin, which reacts with ferric ammonium citrate producing a dark precipitate and green-grey colonies with beige halos. If colonies of enterococci or staphylococci do grow on this medium they can be easily recognized, since they utilise mannitol and produce yellow colonies and haloes, contrasting with the cherry-red colour of medium.
However, when there are many *Listeria* colonies, the entire medium darkens, which can cause interference in differentiation. In these cases it is advisable to perform the inoculation with a more diluted sample.

Technique:
Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.
Reconstitute the vial with the 6 ml sterile distilled water in aseptic conditions and add it to 500 ml of sterilized PALCAM agar base cooled to 50ºC. Do not overheat once suplemented.
Pour the complete medium into Petri dishes and, once solidified on a flat surface, spread the plates by streaking methodology or by spiral method.
Incubate the plates in aerobic atmosphere at 37 ± 1ºC for 44 ± 4h.
(Incubation times longer than those mentioned above or different incubation temperatures may be requied depending on the sample, on the specifications,...)
After incubation, enumerate all the colonies that have appeared onto the surface of the agar, observing any blackening of the medium due to esculin hydrolysis, typical for Listeria strains.

Presumptive isolation of *Listeria sp.* must be confirmed by further microbiological and biochemical tests.

Precautions
For in vitro diagnostic use. Do not reuse. For professional use only.
Do not use the product if it shows evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Quality control

Physical/Chemical control

Color : Orange

Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Isolation by loop spreading

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 37 ºC ± 1, reading after 44 ± 4h

Microbiological control according to the current version of the ISO 11133:2014/A1:2018.

Microorganism	Growth
L. monocytogenes ATCC® 13932, WDCM 00021	Good - Esculin Positive reaction
Escherichia coli ATCC® 25922, WDCM 00013	Inhibited
Enterococcus faecalis ATCC® 29212, WDCM 00087	Inhibited
L. monocytogenes ATCC® 7644	Good - Esculin Positive reaction

Sterility control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.
Add 5mL of the sample to 100 mL of TSB and to 100 mL Thioglycollate.

Bibliography

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. 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.
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