

## 77408 *Listeria* mono Differential Agar, Base (*Listeria monocytogenes* Differential Agar, Base; Agar *Listeria* Ottaviani & Agosti)

### NutriSelect plus

*L. mono* differential Agar has been recommended by ISO Committee for the selective and differential isolation of *Listeria monocytogenes*.

### Composition:

Ingredients	Grams/Litre
Meat peptone	18.0
Casein enzymic hydrolysate	6.0
Yeast extract	10.0
Sodium pyruvate	2.0
Glucose	2.0
Magnesium glycerophosphate	1.0
Magnesium sulphate	0.5
Sodium chloride	5.0
Lithium chloride	10.0
Disodium hydrogen phosphate anhydrous	2.5
Chromogenic substrate	0.05
Agar	15.0
Final pH 7.2 +/- 0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Appearance: Light beige coloured, homogeneous, free flowing powder.  
Colour and Clarity: Light amber coloured, opalescent gel forms in petri plate.

### Directions:

Suspend 36.0 g in 465 ml distilled water. Boil gently to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 min. Cool to 45-50°C. Aseptically add sterile contents of 1 vial of *Listeria* mono Enrichment Supplement I (Cat. No. 03708) and sterile rehydrated contents of *Listeria* mono Selective Supplement (Cat. No. 40784). Mix well and pour into sterile petri plates.

### Principle and Interpretation:

*Listeria* mono Differential Agar is based on the formulation of Ottaviani and Agosti (3,4) used for isolation and cultivation of *Listeria monocytogenes* from foodstuffs and other materials. This formulation was adopted and integrated into the ISO Method 11290-2. Meat peptone, casein enzymic hydrolysate and yeast extract provide essential growth nutrients like vitamin, amino acids and other nitrogenous compounds. Sodium pyruvate and Magnesium glycerophosphate protects injured cells, helps recovery of *Listeria* and enhances growth, while D-glucose is the fermentable sugar. Magnesium sulfate provides necessary ions for the metabolism of *Listeria*. As buffering, substance Disodium hydrogen phosphate is used, and the osmotic balance is given by the sodium chloride. Agar is the solidifying agents. Nalidixic acid, ceftazidime, Amphotericin B, polymyxin B in the supplements and lithium chloride in the medium inhibit bacterial or fungus growth. The chromogenic substrate is used for the detection of the  $\beta$ -glucosidase enzyme present in all common *Listeria* spp. and a few species of enterococci and bacilli. Phosphatidylinositol added with the supplement can be cleaved by specific phospholipase present in all *Listeria monocytogenes* and some strains of *Listeria ivanovii*. When phosphatidylinositol is cleaved it gives a precipitate which can be seen as opaque halo around the colonies.



On this medium, colonies of *Listeria monocytogenes* give a blue-green colour with an opaque halo while the others *Listeria* spp. appear as blue-green colonies without any halo.

Cultural characteristics after 24-48 hours at 35-37°C

Organisms (ATCC, <b>WDCM</b> )	Inoculum (CFU)	Growth	Recovery	Colour of Colony	PIPLC activity*
<i>Listeria monocytogenes</i> (19112)	50-100	+++	≥50%	greenish blue	+
<i>Listeria innocua</i> (33090, <b>00017</b> )	50-100	+++	≥50%	greenish blue	-
<i>Listeria ivanovii</i> (19119, <b>00018</b> )	50-100	+++	≥50%	greenish blue	+
<i>Listeria grayi</i> (19120)	50-100	+++	≥50%	greenish blue	-
<i>Listeria seeligeri</i> (35967)	50-100	+++	≥50%	greenish blue	-
<i>Listeria welshimeri</i> (35897)	50-100	+++	≥50%	greenish blue	-
<i>Escherichia coli</i> (25922, <b>00013</b> )	≥10 <sup>4</sup>	-	0%	-	-
<i>Enterococcus faecalis</i> (29212, <b>00087</b> )	≥10 <sup>4</sup>	-	0%	-	-
<i>Pseudomonas aeruginosa</i> (27853, <b>00025</b> )	≥10 <sup>4</sup>	-	0%	-	-
<i>Candida albicans</i> (10231, <b>00054</b> )	≥10 <sup>4</sup>	-	0%	-	-

\* = phosphatidylinositol phospholipase C activity (opaque halo around the colony)

#### References:

1. ISO 11290-1:2017, Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method
2. ISO 11290-2:2017, Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 2: Enumeration method
3. F. Ottaviani, M. Ottaviani, M. Agosti, Esperienza su un agar selettivo e differenziale per *Listeria monocytogenes*, *Industrie Alimentari* 36, 1-3 (1997)
4. F. Ottaviani, M. Ottaviani, M. Agosti, Differential agar medium for *Listeria monocytogenes*, *Quinper Froid Symposium Proceedings*, p.6, A.D.R.I.A. Quinper, 16-18. June (1997)
5. Fraser and Sperber, Rapid detection of *Listeria* spp. in food and environmental samples by esculin hydrolysis. *J. Food Prot.* 51, 762-765 (1988)
6. Donnelly and Baigent, Method for flow cytometric detection of *Listeria monocytogenes* in milk, *Appl. Environ. Microbiol.*, 52, 689-695 (1986)
7. W.H. Lee, D. McClain, Improved *Listeria monocytogenes* Selective Agar. *Applied and Environmental Microbiology*, 52, 1215-1217 (1986)
8. G. Vlaemynck, V. Lafargue, S. Scotter, Improvement of *Listeria monocytogenes* by the application of ALOA, a diagnostic, chromogenic isolation medium, *J. Appl. Microbiol.*, 88, 430 (2000)



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