Technical Information

L. mono Confirmatory Agar Base

Product Code: DM 2552

Application: - L. mono Confirmatory Agar Base is recommended for the selective and differential isolation of *Listeria monocytogenes* from clinical and food specimens.

Composition**

Ingredients	Gms / Litre
Special peptone	30.000
Yeast extract	6.000
Sodium chloride	5.000
Lithium chloride	10.000
Disodium hydrogen phosphate anhydrous	2.500
B.C. indicator	8.600
alpha-Methyl D-mannoside	3.000
Agar	12.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

L. mono Confirmatory Agar Base is a modification of the formulation of Ottoviani and Agosti (1, 2) for the selective and differential isolation of *Listeria monocytogenes*.

Listeria monocytogenes is a gram-positive foodborne human pathogen responsible for serious infections in pregnant women that may ultimately result in abortion, stillbirth, birth of a child with neonatal listeriosis and meningitis or primary bacteremia in adults and juveniles. The pathogenicity of *Listeria ivanovii* for humans is uncertain. Since *L. monocytogenes* and *L.innocua* have similar biochemical properties, they cannot be differentiated on traditional media (PALCAM, Oxford).

Special peptone and yeast extract supply nitrogen sources and provide essential nutrients required for the growth of Listeria @. #-Methyl-D-mannoside act as a fermentable carbohydrate. Lithium chloride and added selective supplements (MS2212 and MS2213) inhibit accompanying microflora and thus enhance the selectivity of the medium for *Listeria* species. Sodium chloride maintains the osmotic equilibrium and disodium hydrogen phosphate buffers the medium. Differentiation of *L. monocytogenes* from other *Listeria* species is based on phosphatidylinositol-specific phospholipase C (PIPLC) activity and fermentation of #-Methyl D-mannoside. Phospholipase C enzyme is an important virulence factor and is specific to only *L. monocytogenes* and *L.ivanovii*. Phospholipase C enzyme produced by *L.monocytogenes* and *L.ivanovii* hydrolyses the p urified substrate (MS2227) added to the medium and results in the formation of an opaque halo around the colonies.

Differentiation between *L.monocytogenes* and *L.ivanovii* is done on the basis of alpha-Methyl D-mannoside utilization and PIPLC activity. *L.monocytogenes* ferments alpha-Methyl D-mannoside forming yellow coloured colonies with halo whereas *L.ivanovii* fails to ferment alpha-Methyl D-mannoside and therefore forms purple coloured colonies with halo. Other *Listeria* species do not exhibit PIPLC activity and therefore they form either purple or yellow coloured colonies without halo.

Methodology

Suspend 38.5 grams of dehydrated powder media in 470 ml distilled water. Mix thoroughly & heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile rehydrated contents of 1 vial of
L. mono Selective Supplement I (MS2212) and 1 vial of L. mono Selective Supplement II (MS2213). For enrichment, add sterile contents of 1 vial of L. mono Enrichment Supplement II (MS2227) Mix well and pour into sterile Petri plates.

Warning: Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, immediately wash with plenty of water.

Quality Control

Appearance
Beige to purple homogeneous free flowing powder

Gelling
Firm, comparable with 1.2% Agar gel.

Colour and Clarity
Purple coloured, opalescent gel forms in Petri plates

Reaction
Reaction of 7.7% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range
7.00-7.40

Cultural Response
DM2552: Cultural characteristics observed with added supplements, L.mono Selective supplementl (MS2212), L.mono Selective Supplement II (MS2213) and L.mono Enrichment Supplement II (MS2227), after an incubation at 35-37°C for 24-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	PIPLC activity
Cultural Response					
Candida albicans ATCC 10231	>=10^3	inhibited	0 %	-	-
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0 %	-	-
Escherichia coli ATCC 25922	>=10^3	inhibited	0 %	-	-
Listeria innocua ATCC 33090	50-100	luxuriant	>=50%	yellow	negative
Listeria grayi ATCC 19120	50-100	luxuriant	>=50%	yellow	negative
Listeria ivanovii ATCC 19119	50-100	luxuriant	>=50%	light purple	positive, opaque halo around the colony exhibiting phosphatidylinositol specific phospholipase activity

Listeria monocytogenes ATCC 19112	50-100	luxuriant	>=50%	yellow	positive, opaque halo around the colony exhibiting phosphatidylinositol specific phospholipase activity
Listeria seeligeri ATCC 35967	50-100	luxuriant	>=50%	light purple	negative
Listeria welshimeri ATCC 43549	50-100	luxuriant	>=50%	yellow	negative
Pseudomonas aeruginosa ATCC 27853	>=10^3	inhibited	0%	-	-

Storage and Shelf Life

Dried Media: Store at 10-30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Ottaviani F., Ottaviani M., and Agosti M. (1997 a), Industrie Alimentari 36, 1-3.
2. Ottaviani F., Ottaviani M., and Agosti M. (1997 b), Quimper Froid Symposium Proceedings p.6, A.D.R.I.A. Quimper, France, 16-18 June 1997.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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