L. Mono Differential Agar Base

Recommended for the selective and differential isolation of *Listeria monocytogenes* based on PIPLC activity.

M1540

Composition **

Ingredients	Grams/Litre
HM peptone#	18.00
Tryptone	6.00
Yeast extract	10.00
Sodium pyruvate	2.00
Glucose (Dextrose)	2.00
Magnesium glycerophosphate	1.00
Magnesium sulphate	0.50
Sodium chloride	5.00
Lithium chloride	10.00
Disodium hydrogen phosphate	2.50
Chromogenic substrate	0.05
Agar	15.00

Final pH (at 25°C) 7.2 ± 0.2
** Formula adjusted, standardized to suit performance parameters
# Equivalent to Meat peptone

Directions

Suspend 36.02 grams in 460 ml distilled water. 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 (FD212) and L .mono Selective Supplement II (FD213). For enrichment add sterile content of L. mono Enrichment Supplement I (FD214). Mix well and pour into sterile Petri plates.

Principle and Interpretation

L. mono Differential Agar Base is based on the formulation of Ottoviani and Agosti (2, 3) for the selective and differential isolation of *Listeria monocytogenes* from food and animal feeds which is adopted by ISO Committee (1).

HM peptone, Tryptone, yeast extract and sodium pyruvate provide essential growth nutrients and nitrogenous, carbonaceous compounds, long chain amino acids and vitamin B complex. Glucose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added selective supplements (FD212 and FD213) inhibit accompanying microflora and allow the growth of *Listeria* species. *Listeria* species hydrolyse the chromogenic substrate which produces greenish-blue coloured colonies. Differentiation of *Listeria monocytogenes* from other *Listeria* species is based on phosphatidylinositol-specific phospholipase C (PIPLC) activity. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *Listeria monocytogenes* colonies.

Type of specimen

Food samples

Specimen Collection and Handling

For Food samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4).

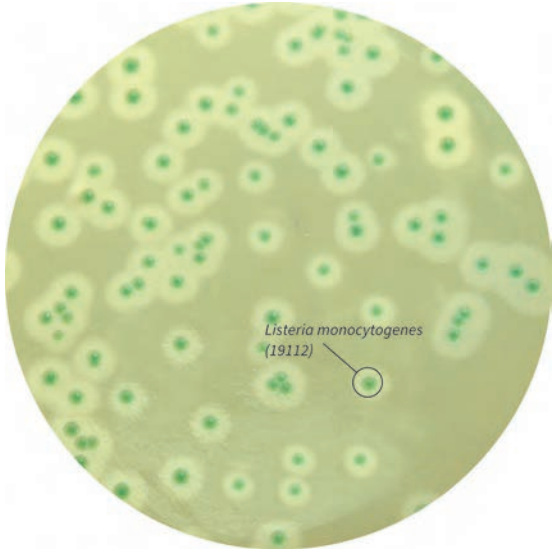
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations

1. Due to variable nutritional requirements, some strains may show poor growth on this medium.
2. Further biochemical tests must be carried out to differentiate between *L. monocytogenes* and *L. ivannovi*.



M1540 L. Mono Differential Agar Base

HiCromeVeg™ Single Streak Rapid Differentiation Series *Freedom from BSE / TSE worries*

L. Mono Differential Agar Base(M1540) is also available as L. Mono Differential HiVeg™ Agar Base (MV1540) & L. Mono Differential HiCynth™ Agar Base (MCD1540) wherein all the animal origin nutrients have been replaced by vegetable based nutrients and chemically defined peptones respectively.

L. Mono Differential Agar Base

Recommended for the selective and differential isolation of *Listeria monocytogenes* based on PIPLC activity.

M1540

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the recommended temperature.

Quality Control

Appearance of Powder : Cream to yellow coloured, homogenous free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Light amber coloured, opalescent gel forms in Petri plates.

Reaction : Reaction of 7.2% w/v aqueous solution at 25°C.

pH : 7.2 ± 0.2

Cultural Response : Cultural characteristics observed withadded L. mono Selective Supplement I(FD212), L. mono Selective Supplement II (FD213) and L. mono Enrichment Supplement I (FD214) after an incubation at 35-37°C for 24-48 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of the Colony	PIPLC activity #
Candida albicans (10231) (00054*)	≥10^3	inhibited	0%	-	-
Enterococcus faecalis (29212) (00087*)	≥10^3	inhibited	0%	-	-
Escherichia coli (25922) (00013*)	≥10^3	inhibited	0%	-	-
Listeria innocua (33090) (00017*)	50-100	luxuriant	≥50%	greeish-blue	-
Listeria grayi (19120)	50-100	luxuriant	≥50%	greeish-blue	-
Listeria ivanovii (19119) (00018*)	50-100	luxuriant	≥50%	greeish-blue	+
Listeria monocytogenes (19112)	50-100	luxuriant	≥50%	greeish-blue	+
Listeria seeligeri (35967)	50-100	luxuriant	≥50%	greeish-blue	-
Listeria welshimeri (43549)	50-100	luxuriant	≥50%	greeish-blue	-
Psedumonas aeruginosa (27853) (00025*)	≥10^3	inhibited	0%	-	-

Key : * : Corresponds to WDCM number

PIPLC activity # : opaque halo around the colony exhibiting phosphatidylinositol - specific phospholipase activity.

Storage and Shelf-life

Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

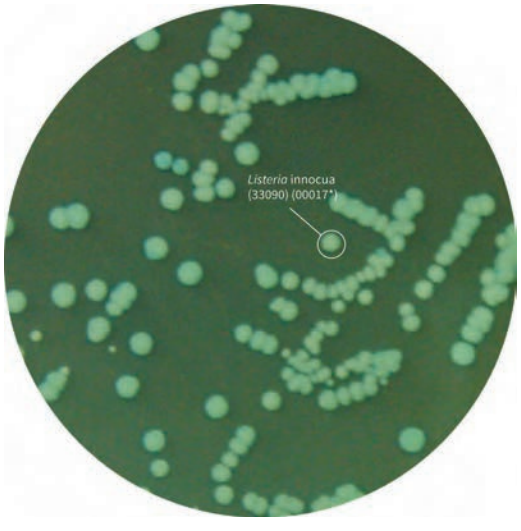
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5, 6).

References

1. Draft Amendment ISO 11290-2:1996/DAM 1.
2. Ottaviani F., Ottaviani M., and Agosti M. (1997 a), Industrie Alimentari 36, 1-3.
3. 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.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1



M1540 L. Mono Differential Agar Base