HiCrome™ L. mono Differential Agar Base

Recommended for the selective and differential isolation, enumeration and identification of *Listeria monocytogenes* and *Listeria species* based on PCPLC activity.

M2009

Composition **

Ingredients	Grams/Litre
Peptone	15.00
Tryptone	6.00
Yeast extract	10.00
Sodium pyruvate	2.00
Maltose	4.00
Magnesium glycerophosphate	1.00
Magnesium sulphate	0.50
Sodium chloride	5.00
Lithium chloride	5.00
Disodium hydrogen phosphate	2.50
Chromogenic substrate	2.20
Agar	14.00

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

Directions

Suspend 33.60 grams in 480 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile contents of 1 vial of Lecithin solution (FD332) and sterile rehydrated contents of Modified L.mono Selective Supplement (FD333). Mix well and pour into sterile Petri plates.

Principle and Interpretation

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 (2). The pathogenicity of *Listeria ivanovii* for humans is uncertain. Since *L.mono*cytogenes and *L.innocua* have similar biochemical properties, they cannot be differentiated on traditional media (PALCAM, Oxford). HiCrome *L.mono* Differential Agar Base is based on for the selective and differential isolation of *Listeria* species on the basis of utilization of chromogenic substrate and lecithinase activity [Phosphotidylcholine hospholipase C (PCPLC)] (3). PI-PLC and PC-PLC, the major virulence factors, are only produced by pathogenic *L. monocytogenes* and *Listeria ivanovii* (1)

Peptone, tryptone, yeast extract and sodium pyruvate provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and essential growth nutrients . Maltose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added selective supplement (FD333) inhibit accompanying microflora and allow the growth of *Listeria* species. *Listeria* species hydrolyse the chromogenic substrate and produces green coloured colonies. Lecithin solution (FD332) helps in detecting PCPLC activity. Differentiation of *Listeria* species is based on phosphatidylcholine phospholipase C (PCPLC) activity. *L. monocytogenes* and *L.ivanovii* exhibits PCPLC activity which is seen as opaque halo around the colony.

Type of specimen

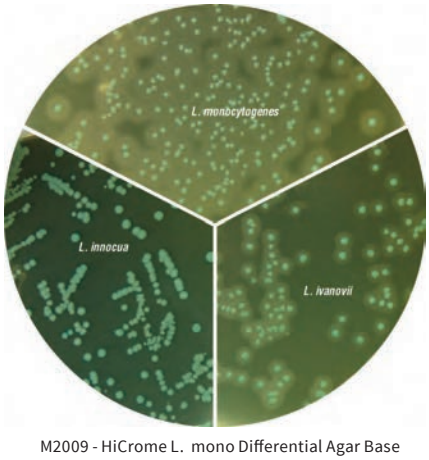
Food samples

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



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Limitations

1. Due to variable nutritional requirements, some strains may show poor growth on this medium.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.

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 homogeneous free flowing powder

Gelling : Firm, comparable with 1.4% Agar gel

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

Reaction : Reaction of 6.72% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH : 7.00-7.40

Cultural Response : Cultural characteristics observed with added sterile Modified L.mono Selective Supplement (FD333) and Lecithin solution (FD332) after an incubation at 35 - 37°C for 24 - 48 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony	PIPLC Activity#
Enterococcus faecalis (29212) (00087*)	≥10^3	inhibited	0%	-	-
Listeria innocua (33090) (00017*)	50-100	luxuriant	≥50%	greeish-blue	negative
Listeria ivanovii (19119) (00018*)	50-100	luxuriant	≥50%	greeish-blue	positive#
Listeria monocytogenes	50-100	luxuriant	≥50%	greeish-blue	positive#

Key: # : opaque halo around the colony exhibiting phophatidylcholine phospholipase acivity
* : corresponds to WDCM numbers

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

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