



L. mono Differential Agar Base

M1540

L. mono Differential Agar Base has been recommended for the selective and differential isolation of *Listeria monocytogenes*.

Composition**

| Ingredients | Gms / Litre |
|---------------------------------------|-------------|
| Meat peptone | 18.000 |
| Casein enzymic hydrolysate | 6.000 |
| Yeast extract | 10.000 |
| Sodium pyruvate | 2.000 |
| Glucose | 2.000 |
| Magnesium glycerophosphate | 1.000 |
| Magnesium sulphate | 0.500 |
| Sodium chloride | 5.000 |
| Lithium chloride | 10.000 |
| Disodium hydrogen phosphate anhydrous | 2.500 |
| Chromogenic substrate | 0.050 |
| Agar | 15.000 |
| Final pH (at 25°C) | 7.2±0.2 |

**Formula adjusted, standardized to suit performance parameters

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 contents of 1 vial of L. mono Enrichment Supplement I (FD214) and sterile rehydrated contents of L. mono Selective Supplement I (FD212), L. mono Selective Supplement II (FD213). Mix well and pour into sterile Petri plates.

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

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

Meat peptone, casein enzymic hydrolysate, yeast extract and sodium pyruvate provide essential growth nutrients and nitrogenous substances. 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 green 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.

Quality Control

Appearance

Cream to yellow homogeneous 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

pH

7.00-7.40

Cultural Response

Cultural characteristics observed with added sterile 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.

Cultural Response

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of Colony | PIPLC activity |
|------------------------------------------|------------------|-----------|----------|------------------|---------------------------------------------------------------------------------------------------------|
| Cultural Response | | | | | |
| <i>Candida albicans</i> ATCC 10231 | ≥10 ³ | inhibited | 0% | | |
| <i>Enterococcus faecalis</i> ATCC 29212 | ≥10 ³ | inhibited | 0% | | |
| <i>Escherichia coli</i> ATCC 25922 | ≥10 ³ | inhibited | 0% | | |
| <i>Listeria innocua</i> ATCC 33090 | ≥10 ³ | luxuriant | ≥50% | greenish-blue | negative |
| <i>Listeria grayi</i> ATCC 19120 | 50-100 | luxuriant | ≥50% | greenish-blue | negative |
| <i>Listeria ivanovii</i> ATCC 19119 | 50-100 | luxuriant | ≥50% | greenish-blue | positive, opaque halo around the colony exhibiting phosphatidylinositol specific phospholipase activity |
| <i>Listeria monocytogenes</i> ATCC 19112 | 50-100 | luxuriant | ≥50% | greenish-blue | positive, opaque halo around the colony exhibiting phosphatidylinositol specific phospholipase activity |
| <i>Listeria seeligeri</i> ATCC 35967 | 50-100 | luxuriant | ≥50% | greenish-blue | negative |
| <i>Listeria welshimeri</i> ATCC 43549 | 50-100 | luxuriant | ≥50% | greenish-blue | negative |
| <i>Pseudomonas aeruginosa</i> ATCC 27853 | ≥10 ³ | inhibited | 0% | | |

Storage and Shelf Life

Store dehydrated powder and the prepared medium at 2-8° C in tightly closed container . Use before expiry date on the label.

Reference

- Ottaviani F., Ottaviani M., and Agosti M. (1997 a), Industrie Alimentari 36, 1-3.
- 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.
- Draft Amendment ISO 11290-2:1996/DAM 1.

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