

TM 2148 – L.MONO BLOOD AGAR BASE

INTENDED USE

For the specific isolation and cultivation of *Listeria* species from food and environmental samples.

PRODUCT SUMMARY AND EXPLANATION

L. monocytogenes is a gram positive, facultatively anaerobic rod shaped bacteria. It can grow under refrigerated condition and therefore is a major concern to the food industry. The recovery of *Listeria* is very low from food and environmental samples, hence it requires enrichment and then further isolation. Various selective and differential media have been proposed for the detection of *Listeria* species in particular *L. monocytogenes*.

L.mono Blood Agar Base was developed by Johanson and Kankare for the isolation of *Listeria* species. It uses Tryptone Soya Agar as a base with the addition of lithium chloride as a selective agent. This medium with the addition of 5% w/v sterile defibrinated sheep blood helps in the differentiation of haemolytic and pathogenic *Listeria* species which includes *L. monocytogenes*, *L. seeligeri* and *L. ivanovii* from non-haemolytic and non-pathogenic species which include *L. innocua*, *L. grayi* and *L. welshimeri*.

COMPOSITION

| Ingredients | Gms / Ltr |
|------------------|-----------|
| Tryptone | 15.000 |
| Soya peptone | 5.000 |
| Sodium chloride | 5.000 |
| Lithium chloride | 10.000 |
| Agar | 15.000 |

PRINCIPLE

This medium consists of Tryptone, soya peptone which provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other growth requirements. Sodium chloride maintains osmotic balance. Lithium chloride, ceftazidime and Polymyxin B sulphate imparts additional selectivity to the medium.

INSTRUCTION FOR USE

- Dissolve 50.0 grams in 970 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi (121°C) for 15 minutes.
- Cool to 45-50°C and aseptically add 5% v/v sterile defibrinated sheep blood and rehydrated contents of two vials of each L. mono selective supplement I (TS 227) and L. mono selective supplement II (TS 228). Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Appearance of prepared medium : Basal medium: Light amber coloured clear to very slightly opalescent gel. After addition of 5%v/v sterile blood : Cherry red opaque gel forms in Petri plates

pH (at 25°C) : 7.3 ± 0.2

INTERPRETATION



Cultural characteristics observed in aerobic atmosphere with added L.mono selective supplement I (TS 227), L.mono selective supplement II (TS 228) and 5%v/v sterile defibrinated blood, after incubation.

| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Recovery | Haemolysis | Incubation Temperature | Incubation Period |
|--|-------|-------------------|----------------|----------|------------------------|------------------------|-------------------|
| <i>Listeria monocytogenes</i> | 19112 | 50-100 | Good-luxuriant | ≥50% | Narrow haemolytic zone | 35-37 °C | 24 - 48 Hours |
| <i>Listeria monocytogenes</i> | 19117 | 50-100 | Good-luxuriant | ≥50% | Narrow haemolytic zone | 35-37 °C | 24 - 48 Hours |
| <i>Listeria monocytogenes subsp. serovar 1</i> | 19111 | 50-100 | Good-luxuriant | ≥50% | Narrow haemolytic zone | 35-37 °C | 24 - 48 Hours |
| <i>Listeria ivanovii subsp. ivanovii serovar 5</i> | 19119 | 50-100 | Good-luxuriant | ≥50% | Wide haemolytic zone | 35-37 °C | 24 - 48 Hours |
| <i>Listeria innocua</i> | 33090 | 50-100 | Good-luxuriant | ≥50% | No haemolysis | 35-37 °C | 24 - 48 Hours |
| <i>Enterococcus faecalis</i> | 29212 | ≥10 ⁴ | Inhibited | 0% | - | 35-37 °C | 24 - 48 Hours |
| <i>Pseudomonas aeruginosa</i> | 27853 | 50-100 | None-poor | 0 - 10 % | - | 35-37 °C | 24 - 48 Hours |
| <i>Proteus mirabilis</i> | 25933 | 50-100 | None-poor | 0 - 10 % | - | 35-37 °C | 24 - 48 Hours |

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 10-25°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

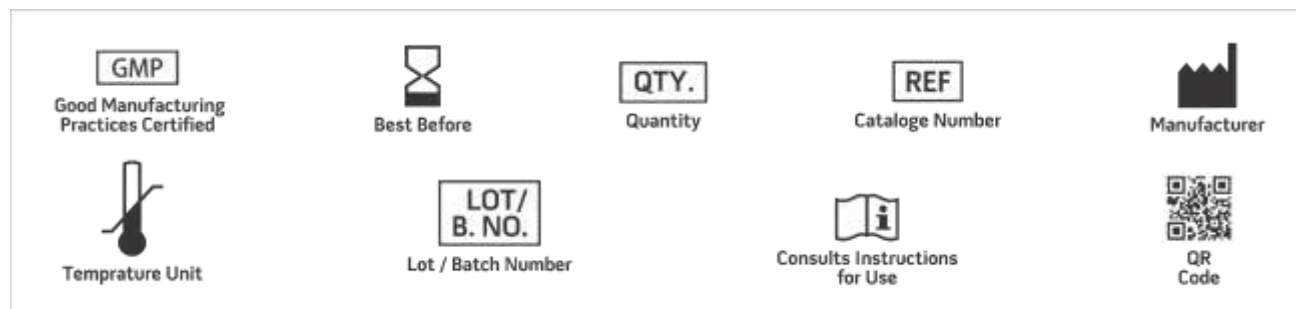
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Bille, J. (1990) Epidemiology of human listeriosis in Europe with special reference to the Swiss outbreak. In Miller, A.J., Smit, J.L. and Somtukti, G.A.(ed.) Foodborne Listeriosis. Elsevier, Amsterdam, pp.71-74.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.



3. 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.
4. Johansson, T., Kankare, M. (1996) Comparison of three selective plating media for the isolation of *Listeria monocytogenes* from fresh broiler cuts. In SLU (ed.) IUFOST Symposium of food Associated pathogens, 6-8 May, 1996, Uppsala, Sweden. Proceedings of the Symposium of Food associated pathogens, pp.228-229.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
Revision: 05 Aug, 2023