

## TM 004- CHROMOGENIC LISTERIA AGAR BASE (ISO 11290-1 & 2:2004, ISO 11133:2014)

### INTENDED USE

For selective identification and differentiation of *Listeria* species.

### PRODUCT SUMMARY AND EXPLANATION

*Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C. Chromogenic Listeria Agar Base (ISO 11290-1:2004) is used for selective identification of *Listeria monocytogenes*. *Listeria* species grow over a pH range of 4.4 - 9.6, and survive in food products with pH levels outside these parameters.

### COMPOSITION

| Ingredients                 | Gms / Ltr |
|-----------------------------|-----------|
| Peptone, Special            | 18.000    |
| Agar                        | 13.000    |
| Yeast Extract               | 10.000    |
| Lithium Chloride            | 10.000    |
| Tryptone                    | 6.000     |
| Sodium chloride             | 5.000     |
| Disodium Hydrogen phosphate | 2.500     |
| Glucose                     | 2.000     |
| Sodium Pyruvate             | 2.000     |
| Magnesium Glycerophosphate  | 1.000     |
| Magnesium Sulphate          | 0.500     |
| X- glucoside                | 0.050     |

### PRINCIPLE

Peptone, Tryptone and Yeast extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride maintains the osmotic balance of the medium. Agar is a solidifying agent. Sodium pyruvate is a source of energy and helps in revival of stressed organisms. Glucose serves as carbon and energy source. Magnesium glycerophosphate and disodium hydrogen phosphate serve as buffering compound. Magnesium sulphate supplies magnesium ion, required for various enzymatic reactions, including DNA replication. Lithium chloride and Chromogenic Listeria Selective Supplement (TS 205) inhibit growth of most gram-positive bacteria, gram negative bacteria, yeasts and moulds. Chromogenic Enrichment Supplement (TS 031) facilitates the detection of phosphatidylinositol phospholipase C (PIPLC), produced by *Listeria monocytogenes*.

This medium is helpful in specific detection of  $\beta$ -glucosidase activity. *Listeria* species hydrolyse the chromogenic substrate X-glucoside in the medium giving blue coloured colonies. Since  $\beta$ -glucosidase activity is specific for *Listeria* species. Other organisms that possess this enzyme are inhibited by the selective agents. Organisms, unable to utilize the chromogenic substrate, give white colonies.

Further, the differential activity is also obtained by lipase C substrate, upon which the specific enzyme for *L. monocytogenes* acts and gives an opaque white halo around *L. monocytogenes* colonies.

### INSTRUCTION FOR USE

- Dissolve 70.05 gm in 970 ml of distilled water.
- Gently heat to boiling with gentle swirling to dissolve the agar completely.
- Sterilize by autoclaving at 15 psi (121°C) for 15 minutes.
- Cool to 45 – 50°C.
- Rehydrate contents of 2 vials of Chromogenic Listeria Selective Supplement (TS 205) in 5 ml distilled water and add aseptically to the molten medium, along with contents of 2 vials of Chromogenic Enrichment Supplement (TS 031).
- Mix well and pour into sterile Petri plates.

### QUALITY CONTROL SPECIFICATIONS

|                               |   |  |
|-------------------------------|---|--|
| Appearance of powder          | : | Light beige colour homogeneous free flowing powder   |
| Appearance of prepared medium | : | Light amber colour, clear to slightly opalescent gel |
| pH (at 25°C)                  | : | 7.2 ± 0.2  |

### INTERPRETATION

Cultural characteristics observed after incubation. Recovery rate is considered 100% for bacteria growth on Soya Agar.

| Microorganism                 | ATCC  | Inoculum (CFU/ml) | Growth    | Colour of colony | Halo     | Recovery | Incubation Temperature | Incubation Period |
|-------------------------------|-------|-------------------|-----------|------------------|----------|----------|------------------------|-------------------|
| <i>Listeria monocytogenes</i> | 19111 | 50-100            | Good      | Blue colour      | Positive | >=50%    | 35 ± 2°C               | 24 - 48 Hours     |
| <i>Listeria innocua</i>       | 33090 | 50-100            | Good      | Blue colour      | Negative | >=50%    | 35 ± 2°C               | 24 - 48 Hours     |
| <i>Enterococcus faecalis</i>  | 19433 | ≥ 1000            | Inhibited | -                | -        | 0%       | 35 ± 2°C               | 24 - 48 Hours     |
| <i>Escherichia coli</i>       | 25922 | ≥ 1000            | Inhibited | -                | -        | 0%       | 35 ± 2°C               | 24 - 48 Hours     |

### PACKAGING

In pack size of 100gm & 500gm bottles.

### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 2-8°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 powder 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. McLain D. and Lee W.H. FSIS Method for the isolation and identification of *Listeria monocytogenes* from processed meat and poultry products. Laboratory Communications number 57. (1989)
2. Ottaviani, F., Ottaviani, M. and Agosti, M (1987) Quimper Froid Symposium Proceedings, P6 A.D.R.I.A Quimper (F) 16-18 June ISO 11290-1:2004 Horizontal method for the detection and enumeration of *Listeria monocytogenes* Part 1: Detection Method.
3. U.S. Food and Drug Administration. Bacteriological analytical manual (online), AOAC International, Gaithersburg, MD. (2003).
4. U.S. Department of Agriculture Food Safety and Inspection Services, Office of Public Health and Science. *Isolation and identification of Listeria monocytogenes from red meat, poultry, egg and environmental Samples.* In Microbiology Laboratory Guidebook, Washington, D.C. (2002).



|   |   |  |   |   |
|---|---|--|---|---|
| <br>GMP<br>Good Manufacturing<br>Practices Certified | <br>Best Before                          | <br>QTY.<br>Quantity                  | <br>REF<br>Cataloge Number | <br>Manufacturer |
| <br>Temperature Unit                                 | <br>LOT/<br>B. NO.<br>Lot / Batch Number | <br>Consults Instructions<br>for Use | <br>QR<br>Code             |   |

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

**\*For Lab Use Only**

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