

# ALOA Agar (Agar Listeria)



Medium used for the isolation and identification of *Listeria monocytogenes*.

\*Equally use with MFDS (MB-A1620K) and Color O.A. Listeria Agar (MB-C1620).

## • CONTENTS (Liter)

|  |        |
|--|--------|
| Enzymatic Digest of Animal Tissues                     | 18.0 g |
| Enzymatic Digest of Casein                             | 6.0 g  |
| Sodium Pyruvate  | 2.0 g  |
| Glucose  | 2.0 g  |
| Magnesium Glycerophosphate                             | 1.0 g  |
| Magnesium Sulfate (Anhydrous)                          | 0.5 g  |
| Sodium Chloride  | 5.0 g  |
| Yeast Extract  | 10.0 g |
| Lithium Chloride                                       | 10.0 g |
| Disodium Hydrogen Phosphate (Anhydrous)                | 2.5 g  |
| 5-Bromo-4-Chloro-3-Indolyl- $\beta$ -D-Glucopyranoside | 0.05 g |
| Agar   | 15.0 g |
| Final pH = 7.2 $\pm$ 0.2 at 25°C                       |        |

## • PROCEDURE

Suspend 72.05 G of powder in 952 mL or 942 mL of distilled or deionized water. Heat to boiling until completely dissolved. Sterilize by autoclave at 121°C for 15 minutes. Cool to 45 - 50°C in water bath. Aseptically add 40 mL of Egg Yolk Emulsion (MB-E1864) to observe lecithinase reaction. Instead of Egg Yolk Emulsion, you may use 2 vials of Phosphatidyl Inositol supplement (MB-P0775). Aseptically add 2 vials of O.A. Listeria Agar supplement (MB-O0774). Mix well. Pour into petri dishes.

### O.A. Listeria Agar supplement

1 vial contents (each vial is sufficient for 500 mL of medium)

|                |          |
|----------------|----------|
| Polymyxin B    | 38350 IU |
| Ceftazidime    | 0.01 g   |
| Nalidixic Acid | 0.01 g   |
| Cycloheximide  | 0.025 g  |

### Phosphatidyl Inositol supplement

1 vial contents (each vial is sufficient for 500 mL of medium)

|                                   |       |
|-----------------------------------|-------|
| L- $\alpha$ -phosphatidylinositol | 1.0 g |
|-----------------------------------|-------|

## • INTERPRETATION

ALOA Agar (Agar Listeria) is a medium used for the isolation and identification of *Listeria monocytogenes*. Enzymatic digest of animal tissues, enzymatic digest of casein and yeast extract provide nitrogenous compounds, vitamins and minerals. Sodium pyruvate and magnesium glycerophosphate increase the recovery of stress cells. Glucose is the fermentable carbohydrate. Magnesium sulfate (anhydrous) provides necessary ions for the metabolism of organisms. Sodium chloride maintains the osmotic balance. Lithium chloride, polymyxin B, ceftazidime, nalidixic acid and cycloheximide are the selective agents. Disodium hydrogen phosphate (anhydrous) is the buffering agent. Listeria hydrolyzes chromogenic substrates that result in Listeria spp. producing green-blue colonies. *Listeria monocytogenes* and *Listeria ivanovii* are then further differentiated by their ability to produce the phospholipase enzyme, lecithinase. This enzyme hydrolyzes the lecithin producing opaque halo around the colony. Agar is the solidifying agent. Egg yolk emulsion helps in differentiation of lecithinase producing colonies which are surrounded by a zone of egg yolk precipitation. Phosphatidylinositol phospholipase C an enzyme produced by *Listeria monocytogenes* can be identified by Phosphatidyl inositol supplement.

## • TECHNIC

Inoculate the specimen using a sterile loop to the medium. Incubate at  $36 \pm 1^{\circ}\text{C}$  for 24 - 48 hours. Refer appropriate references for recommended test procedure.

## • QUALITY CONTROL FOR USE

### Dehydrated medium

Appearance: free-flowing, homogeneous

Color: beige

### Prepared medium

Appearance: opaque

Color: yellow

Incubation conditions:  $36 \pm 1^{\circ}\text{C}$  / 24 - 48 hours

| Microorganism                 | ATCC  | Growth    | Characteristics             |
|-------------------------------|-------|-----------|-----------------------------|
| <i>Listeria monocytogenes</i> | 15313 | good      | green-blue with opaque halo |
| <i>Listeria innocua</i>       | 33090 | good      | green-blue                  |
| <i>Listeria ivanovii</i>      | 19119 | good      | green-blue with opaque halo |
| <i>Enterococcus faecalis</i>  | 29212 | inhibited | -                           |
| <i>Escherichia coli</i>       | 25922 | inhibited | -                           |
| <i>Pseudomonas aeruginosa</i> | 27853 | inhibited | -                           |

## • STORE

The powder is very hygroscopic. Store the powder at  $2 - 8^{\circ}\text{C}$ , in a dry environment, in its original container tightly closed and use it before the expiry date on the label. Store prepared medium at  $2 - 8^{\circ}\text{C}$ .

## • REFERENCES

1. Artault, S., Bind, J.L., Delaval, Y., Dureuil, N., Gillard, N., (2000) AFNOR Validation of the ALOA method for the detection of *Listeria monocytogenes* in foodstuffs. Colloque de la Societe Francaise de Microbiologie, Paris 19-20 Octobre, 2000.
2. ISO 11290 1/2 (Draft, May 2002) Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Listeria monocytogenes*.
3. Ottaviani, F., Ottaviani, M., Agosti, M., (1997) Esperienze su un agar selettivo e differenziale per *Listeria monocytogenes*. Industrie alimentari, XXXVI, luglio-agosto, 888.
4. Refer to the MFDS.

## • PACKAGE

|   |       |
|---|-------|
| Cat. No : MB-A1620<br>ALOA Agar (Agar Listeria) | 500 G |
|---|-------|