Chromogenic Listeria Differential Supplement

PRODUCT INFORMATION

N001-5g - Nalidixic Acid, Powder, 5g

N001-25g - Nalidixic Acid, Powder, 25g

N001-100g - Nalidixic Acid, Powder, 100g

P007-1MU - Polymyxin B Sulfate, Powder, 1MU

P007-10MU - Polymyxin B Sulfate, Powder, 10MU

P007-100MU - Polymyxin B Sulfate, Powder, 100MU

C059-1g - Ceftazidime Pentahydrate, Powder, 1g

C059-5g - Ceftazidime Pentahydrate, Powder, 5g

A007-100mg - Amphotericin B, Powder, 100mg

A007-250mg - Amphotericin B, Powder, 250mg

A007-1g - Amphotericin B, Powder, 1g

A007-5g - Amphotericin B, Powder, 5g

DESCRIPTION

Chromogenic Listeria Agar (ISO) is a medium for isolation, enumeration and presumptive identification of *Listeria* species and *Listeria monocytogenes* from food samples.

BACKGROUND

Nalidixic acid is the first of the synthetic quinolone antibiotics. Nalidixic acid is effective against both gram-positive and gram-negative bacteria. In lower concentrations, it acts in a bacteriostatic manner; that is, it inhibits growth and reproduction. In higher concentrations, it is bactericidal, meaning that it kills bacteria instead of merely inhibiting their growth.

Cycloheximide is widely used in biomedical research to inhibit protein synthesis in eukaryotic cells studied in vitro (i.e. outside of organisms). Its effects are rapidly reversed by simply removing it from the culture medium.

Mechanism of action

Cycloheximide is an inhibitor of protein biosynthesis in eukaryotic organisms, produced by the bacterium Streptomyces griseus. Cycloheximide exerts its effect by interfering with the translocation step in protein synthesis (movement of two tRNA molecules and mRNA in relation to the ribosome) thus blocking translational elongation.

APPLICATION IN CHROMOGENIC LISTERIA AGAR (ISO)

Listeria monocytogenes is the most common pathogenic *Listeria* spp. and has been shown to be pathogenic to both man and animals. Some *Listeria ivanovii* strains also possess these enzymes and, although *Listeria ivanovii* are primarily pathogenic to animals, there are strains which have been shown to cause infection in humans. Studies have shown this medium to be superior to PALCAM medium for the isolation of *Listeria monocytogenes*.

Chromogenic Listeria Agar (ISO) uses the chromogen X-glucoside for presumptive identification of *Listeria* spp. This chromogen is cleaved by ß-glucosidase, which is common to all *Listeria* species. Other organisms that possess this enzyme, such as *enterococci*, are inhibited by the selective agents within the medium: lithium chloride, polymyxin B and nalidixic acid, whilst amphotericin inhibits the growth of yeasts and moulds that may be present in the sample. *Listeria monocytogenes* and pathogenic *Listeria ivanovii* are then further differentiated by their ability to produce the phospholipase enzymes PIPLC and PCPLC which hydrolyse phosphotidyliositol or lethicin in the medium, producing an opaque white halo around the colony.

This medium is designed to identify *Listeria* spp. based on their utilisation of a chromogenic substrate. Chromogenic Listeria Agar (ISO), when used with Chromogenic Listeria Selective Supplement (ISO) and Chromogenic Listeria Differential Supplement (ISO) following the manufacturer's instructions, conform to the formulation described by Ottaviani and Agosti (ALOA) in ISO 11290-1:1997 (Incorporating Amendment No.1). This ALOA formulation incorporates phosphatidylinositol so that phosphatidylinositol phospholipase C (PIPLC), produced by *Listeria monocytogenes*, is detected. Alternatively, adding lecithin (Modified Chromogenic Listeria Differential Supplement) instead of phosphatidylinositol means that phosphatidylcholine phospholipase

C (PCPLC) activity can be detected. Both PIPLC and PCPLC are associated with the virulence of *Listeria* and, therefore, detection of either enzyme is a useful indicator of pathogenicity.

Content concentrations

Typical Formula*	mg/litre
Chromogenic Listeria Agar (ISO)	
Enzymatic digest of animal tissues	18
Enzymatic digest of casein	6
Sodium pyruvate	2
Glucose	2
Magnesium glycerophosphate	1
Magnesium sulphate (anhydrous)	0.5
Sodium chloride	5
Yeast extract	10
Lithium chloride	10
Disodium hydrogen phosphate (anhydrous)	2.5
X-glucoside chromogenic mix	0.05
Agar	12
Final pH 7.2 ± 0.2 @ 25°C	
Chromogenic Listeria Selective Supplement (ISO)	
Nalidixic acid	20
Polymyxin B	76,700 IU
Ceftazidime	20
Amphotericin	10
Chromogenic Listeria Differential Supplement (ISO)†	
L-α-phospotidylinositol solution	40.0ml
Modified Chromogenic Listeria Differential Supplement†	
Lecithin solution	40.0ml

* Adjusted as required to meet performance standards

† Please use Chromogenic Listeria Differential Supplement (ISO) or Modified Chromogenic Listeria Differential Supplement in accordance with method being followed

Table 1 - Typical Formula for Chromogenic Listeria Agar (ISO), Chromogenic Listeria Selective Supplement (ISO), Chromogenic Listeria Differential Supplement (ISO) and Modified Chromogenic Listeria Differential Supplementt

METHOD

Preparation

Modified ISO Formulation

Suspend appreciate mount of Chromogenic Listeria Agar (ISO) in distilled water. Mix well and sterilize by autoclaving at 121°C for 15 minutes. Cool the medium to 46°C and add Chromogenic Listeria Selective Supplement (ISO), reconstituted as directed and Modified Chromogenic Listeria Differential Supplement. Mix well and pour into sterile Petri dishes.

ISO Formulation

Suspend appreciate mount of Chromogenic Listeria Agar (ISO) Base in distilled water. Mix well and sterilise by autoclaving at 121°C for 15 minutes. Cool the medium to around 50 °C, and add Chromogenic Listeria Selective Supplement (ISO), re-suspended as directed, and Chromogenic Listeria Differential Supplement (ISO). Mix well and pour into sterile Petri dishes.

Protocol

Chromogenic Listeria Agar (ISO) can be used following a variety of enrichment procedures i.e. ISO, NMKL, FDA, AFNOR (UNI 03/04 - 04/054), etc.

The following method is a summary of ISO 11290-1:1997 (Incorporating Amendment No. 1):

1. Add 25 g of food sample to 225 ml of half fraser broth and stomach for a minimum of 30 seconds to mix the sample.

2. Incubate the broth without agitation at 30°C for 24 ± 3 hours.

3. Gently agitate the bag then, using a microbiological loop inoculate onto Chromogenic Listeria Agar (according to Ottaviani and Agosti) and a second selective medium (e.g. PALCAM Agar). Incubate at 37°C for 24h ± 3h, and if necessary for an additional 24h ± 3h (PALCAM Agar should be incubated under micro-aerobic conditions for best results).

4. Examine the PALCAM plate for black colonies and the chromogenic listeria agar plate for blue colonies with and without halos.

5. From the same incubated half fraser broth remove 0.1 ml and inoculate into 10 ml of Fraser broth. Incubate at 37°C for 48h ± 3h and then repeat Steps 3 & 4 followed by step 6.

6.Confirm presumptive colonies on the agar plates as Listeria monocytogenes or Listeria spp. by appropriate methods - refer to ISO 11290-1:1997 (Incorporating Amendment No.1)

Quality control

Positive control:

Listeria monocytogenes ATCC®7644: Good growth: blue/green plus halo

Listeria innocua ATCC®33090: Good growth: blue/green no halo

Negative control:

Enterococcus feacalis ATCC®29212: Inhibited

REFERENCES

1. ISO 11290-1:1997 (Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of Listeria monocytogenes - part 1, Incorporating Amendment 1.)

2. Cummins, A.J., Fielding, A.K. and McLauchlin, J. (1994) Listeria ivanovii infection in a patient with AIDS. Journal of Infection 28, p89-91