

Modified Listeria Selective Enrichment Supplement

PRODUCT INFORMATION

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

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

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

C001-1g - Cycloheximide, Powder, 1g

C001-5g - Cycloheximide, Powder, 5g

DESCRIPTION

Listeria Enrichment Broth Base with Modified Listeria Selective Enrichment Supplement is a selective enrichment medium for the isolation of *Listeria monocytogenes*.

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 LISTERIA ENRICHMENT BROTH BASE

Listeria Selective Enrichment Medium is based on the formulation described by Lovett et al. and is recommended for the selective enrichment of *Listeria* spe-

cies from food. The enrichment procedure has been shown to recover an inoculum of less than 10 cfu/ml from raw milk.

In order to achieve a higher isolation rate it is recommended that the enrichment broth is subcultured onto Listeria Selective Agar plates after 1, 2 and 7 days. Agello et al., have shown that extending the incubation period to 7 days allows better recovery of environmentally stressed listeria from milk and milk products.

Content concentrations

Typical Formula*	mg/litre
Listeria Enrichment Broth Base	
Tryptone soya broth	30
Yeast extract	6
Final pH 7.3 ± 0.2 @ 25°C	
Modified Listeria Selective Enrichment Supplement	
Nalidixic acid	40
Cycloheximide	50
Acriflavine hydrochloride	10
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Listeria Enrichment Broth Base and Modified Listeria Selective Enrichment Supplement

METHOD

Preparation

Suspend appropriate amount of Listeria Enrichment Broth Base in distilled water. Add the contents of Modified Listeria Selective Enrichment Supplement, reconstituted with 2 ml of distilled water. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C, mix well and distribute into sterile containers in volumes as required.

Protocol

1. Add 25 g or 25 ml samples to 225 ml of Listeria Selective Enrichment Broth. Homogenise if required.
2. Incubate at 30°C for 7 days.
3. Subculture from the Listeria Selective Enrichment Broth onto Listeria Selective Agar plates (see Note) after 1, 2 and 7 days by:
 - (i) Direct plating onto Listeria Selective Agar plates.
 - (ii) Adding 1 ml of the Listeria Selective Enrichment

Broth to 9ml of 0.5% KOH, vortex mixing, and plating onto Listeria Selective Agar plates.

Note:

Suitable Listeria Selective Media are:

1. Listeria Selective Medium.
2. PALCAM Medium

Quality control

Positive control:

Listeria monocytogenes ATCC® 7644: Turbid growth

Negative control:

Enterococcus faecalis ATCC® 29212: Inhibited

REFERENCES

1. Lovett J., Francis D. W. and Hunt J. M. (1987) *Journal of Food Protection* 50. 188-192.
2. Agello G., Hayes P. and Feeley J. (1986) *Abstracts of the Annual Meeting, ASM, Washington DC* p5.

