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

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

Listeria Enrichment Broth Base with 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* species 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	
Listeria Selective Enrichment Supplement	
Nalidixic acid	40
Cycloheximide	50
Acriflavine hydrochloride	15
* Adjusted as required to meet performance standards	

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

METHOD

Preparation

Suspend appreciate amount of Listeria Enrichment Broth Base in distilled water. Add the contents of 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 feacalis 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.

APPLICATION IN BUFFERED LISTERIA ENRICHMENT BROTH

Listeria Selective Enrichment Broth is based on the formulation described by Lovett et al. and is recommended for the enrichment of *Listeria* species in food. Subsequent work has concluded that the enrichment properties can be improved by increasing the buffering capacity of the medium by the addition of potassium di-hydrogen orthophosphate and disodium hydrogen orthophosphate. Buffered Listeria Enrichment Broth is therefore a modification of the original medium.

Content concentrations

Typical Formula*	**mg/litre**
Buffered Listeria Enrichment Broth	
Tryptone soya broth	30
Yeast extract	6
Potassium di-hydrogen orthophosphate	1.35
Disodium hydrogen orthophosphate	9.6
Final pH 7.3 ± 0.2 @ 25°C	
Modified Listeria Selective Enrichment Supplement	
Nalidixic acid	40
Amphotericin B	10
Acriflavine hydrochloride	15
* Adjusted as required to meet performance standards	

Table 2 - Typical Formula for Buffered Listeria Enrichment Broth and Modified Listeria Selective Enrichment Supplement

METHOD

Preparation

Add appreciate mount of Buffered Listeria Enrichment Broth to distilled water and mix well to dissolve. Add the contents of Modified Listeria Selective Enrichment Supplement reconstitued as directed. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C, mix well and aseptically distribute into sterile containers in volumes as required.

Protocol

1. Add 25 g or 25 ml samples to 225ml of Buffered Listeria Enrichment Broth. Homogenise if required.

2. Incubate at 30°C for 48 hours.

3. Subculture from the Buffered Listeria Emrichment Broth onto Listeria Selective Agar plates (See Note) after 24 and 48 hours by:

(i) Direct plating onto Listeria Selective Agar plates.

(ii) Adding 1ml of the Buffered Listeria Enrichment Broth to 9 ml 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

Listeria Selective Enrichment Supplement SR0141 as supplied should be stored at 2-8°C. When stored as directed the reagents are stable until the expiry date printed on the label.

Note:

Suitable Listeria Selective Media are:

1. Listeria Selective Medium.

2. PALCAM Medium

Quality control

Positive control:

Listeria monocytogenes ATCC® 19117: Turbid growth

Negative control:

Enterococcus faecalis ATCC® 29212: Inhibited

REFERENCES

1. Lovett J., Francis D.W. and Hunt J.M. (1987) J. Food Prot. 50. 188-192.

2. Curtis G.D.W., Nichols W.W. and Falla T.J. (1989) Lett. Appl. Micro. 8. 169-172.