Listeria Primary Selective Enrichment Supplement (UVM I) and Listeria Secondary Selective Enrichment Supplement (UVM II)

PRODUCT INFORMATION

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

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

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

DESCRIPTION

Listeria enrichment broth base with Listeria primary selective enrichment supplement (UVM I) and Listeria Secondary Selective Enrichment Supplement (UVM II) is a two step selective enrichment medium for isolation of *Listeria monocytogenes* from meat products.

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.

Mechanism of action

APPLICATION IN LISTERIA ENRICHMENT BROTH BASE

The Listeria Selective Enrichment Media (UVM formulations) are based on the original formulation described by Donnelly and Baigent, and its subsequent modification which reduced the nalidixic acid concentration in both the primary and secondary selective enrichment media and increased the concentration of acriflavine hydrochloride in the secondary selective enrichment medium.

This modification, and the two step selective enrichment method developed (USDA-FSIS method), results in a higher detection rate of *Listeria monocytogenes* from meat products and has the added advantage of only taking 3-4 days.

UVM Broth has been recommended as a primary enrichment broth for recovery of heat-injured *Listeria monocytogenes*.

Care must be taken when using UVM broth with DNA probe methodology because the high salt content of the medium may have an inhibitory effect on detection.

Content concentrations

Typical Formula*	mg/litre
Listeria enrichment broth base	
Proteose peptone	5
Tryptone	5
`Lab-Lemco' powder	5
Yeast extract	5
Sodium chloride	20
Disodium hydrogen phosphate	12
Potassium dihydrogen phosphate	1.35
Aesculin	1
Final pH 7.4 ± 0.2 @ 25°C	
Listeria primary selective enrichment supplement (UVM I)	
Nalidixic acid	20
Acriflavine hydrochloride	12
Listeria Secondary Selective Enrichment Supplement (UVM II)	
Nalidixic acid	20
Acriflavine hydrochloride	25
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Listeria Enrichment Broth Base, Listeria Primary Selective Enrichment Supplement (UVM I) and Listeria Secondary Selective Enrichment Supplement (UVM II)

METHOD

Preparation

Suspend appreciate amount of Listeria Enrichment Broth Base in distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C.

To Prepare Listeria Primary Selective Enrichment Medium (UVM I)

Aseptically add the contents of Listeria Primary Selective Enrichment Supplement (UVM I) reconsituted as directed. Mix well and distribute into sterile containers.

To Prepare Listeria Secondary Selective Enrichment Medium (UVM II)

Aseptically add the contents of Listeria Secondary Selective Enrichment Supplement (UVM II) reconsituted as directed. Mix well and distribute into sterile containers.

Protocol

Primary Enrichment

1. Add 25 g or 25 ml samples to 225 ml of Listeria Primary Selective Enrichment Medium (UVM I). Homogenise in a Stomacher for 2 minutes.

2. Incubate the prepared sample in the Stomacher bag at 30°C.

3. From this bag, carry out the following procedures: After 4 hours incubation, spread 0.2 ml on Listeria Selective Agar plates (see Note).

After 24 hours incubation,

(i) transfer 0.1 ml to 10 ml of Listeria Secondary Enrichment Medium (UVM II), and

(ii) transfer 1 ml to 4.5 ml KOH solution. Vortex mix and within one minute subculture onto Listeria Selective Agar plates. For details of KOH preparation see below.

Secondary Enrichment

4. Incubate the inoculated Listeria Secondary Selective Enrichment Medium (UVM II) at 30°C. See 3(i).

5. After 24 hours incubation,

(i) spread 0.2 ml onto Listeria Selective Agar plates.

(ii) transfer 1 ml to 4.5 ml KOH solution. Vortex mix and within one minute subculture this mixture onto Listeria Selective Agar plates.

Preparation Of KOH Solution

Dissolve 2.5 g of KOH and 20 g of NaCl in 1000 ml of distilled water. Sterilise by autoclaving at 121°C and ensure that the pH is above 12.0 before use.

Note

The Listeria Selective Agar recommended for use in the USDA method is LPM plating medium. However, studies have shown that comparable results can be achieved with Listeria Selective Medium.

Because of the updated of USDA, There is no longer a requirement to treat enrichment culture with potassium hydroxide before plating.

Quality control

Positive control:

Listeria monocytogenes ATCC® 7644: Turbid growth

Negative control:

Enterococcus faecalis ATCC® 29212: No change

REFERENCES

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