Product :
***Listeria* ENRICHMENT BROTH BASE (UVM)**

Also known as
LEB (UVMI)

Specification
Liquid culture medium for the enrichment of *Listeria spp*.

Formula * in g/L
Proteose peptone..... 5,00
Tryptone..... 5,00
Meat extract..... 5,00
Yeast extract..... 5,00
Sodium chloride..... 20,00
Esculin..... 1,00
Disodium phosphate..... 12,00
Dipotassium phosphate..... 1,35

Final pH 7,4 ±0,2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions
Dissolve 54,35 g of powder into 1 L of distilled water. Distribute 500 mL in each container and autoclave at 121ºC for 15 minutes. Cool to 50ºC and then aseptically add the selective supplement to each 500 mL: UVM I for primary enrichment (Art. No. 06-106LYO1) and UVM II/Fraser for secondary enrichment (Art. No. 06-111LYO1).
Note: Prepared medium (broth + supplement) must be kept away from light, since it promote the production of acriflavine-oxidised photo-complexes that repress Listeria growth.

Description
This broth base for the enrichment of *Listeria* is made according to the AOAC modifications of the Vermont University Medium (UVM). Having an increase in acriflavine concentration in the secondary enrichment, and a strong reduction in the amount of nalidixic acid in all stages allows more positive isolations of *Listeria*.

Technique

Primary enrichment

Add 25 g or 25 mL of sample to 225 mL of primary enrichment broth (Broth Base Art. No. 02-472 and UVM I Selective Supplement, Art. No. 06-106LYO1). Homogenize in a Stomacher® for 2 minutes and incubate the mixture at 30 ºC for 24 hours. After the first 4 hours inoculate aliquots of 0,2 mL on Oxford Selective Agar (Art. No. 01-471) plates for isolation purposes.

Secondary enrichment

After 24 hours of primary enrichment, inoculate the secondary enrichment broth (Broth Base Art. No. 02-472 and UVM II/Fraser, Art. No. 06-111LYO1) in a ratio of 1:100. Incubate at 30 ºC. After 4 and 24 hours inoculate aliquots of 0,2 mL on Oxford Selective Agar plates for isolation purposes.

Isolation

Isolation is carried out on the Oxford Selective Base Agar + Selective Supplement, Art. No. 06-127LYO1) plates. Incubate for 24-48 hours at 30-37ºC. It can be advantageousto alkalinize the inoculum before inoculation, by mixing 1 mL of enrichment broth with 5 mL of 0,5% sterile KOH solution.

Necessary supplements

Listeria Selective Supplement for Primary Enrichment (UVM I) (Art. No.06-106LYO1)
Vial contents:
Necessary amount for 500 mL of complete medium.
Nalidixic acid, sodium salt..... 10 mg
Acriflavine.....6 mg
Distilled water (Solvent)

Listeria Selective Supplement for Secondary Enrichment (UVM II / FRASER) (Art. No. 06-111LYO1)
Vial contents:
Necessary amount for 500 mL of complete medium.
Nalidixic acid, sodium salt..... 10,0 mg
Acriflavine.....12,5 mg
Distilled water (Solvent)

Listeria Oxford Selective Supplement (Art. No.06-127LYO1)
Vial contents:
Necessary amount for 500 mL of complete medium.

Cyclohexymide.....0.2000
Colistin sulphate.....0.0100
Acriflavine.....0.0025
Cefotetan.....0.0010
Phosphomycin sodium salt..... 0.0050
Reconstitute the original freeze-dried vial
by adding:
Sterile solvent (50% Ethanol/water).. 9 ml

Quality control

Incubation temperature: 37°C ±1,0 **Incubation time:** 24 ±2 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10^4-10^6 CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Escherichia coli ATCC® 8739	Inhibited	w. antibiotic / recovery in TSA
Enterococcus faecalis ATCC® 19433	Partial inhibition	w. antibiotic / <100 CFU in TSA
Listeria monocytogenes ATCC® 13932	Good	> 10 UFC *Listeria* in A. Listeria Ottaviani Agostini
Listeria monocytogenes ATCC® 35152	Good	> 10 UFC *Listeria* in A. Listeria Ottaviani Agostini
Listeria monocytogenes ATCC® 13932	Good	>10Exp.7 ufc/ml (100 ufc/ml)

Left: Uninoculated tube (Control)
Listeria monocytogenes ATCC 13932
Listeria monocytogenes ATCC 35152

References

· ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. FL.
· McCLAIN, D. & W.H. LEE (1988) Development of USDA-FSIS Method for isolation of Listeria monocytogenes from raw meat and poultry. JAOAC 71:3:660-664.
· VANDERZANT, C & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington. DC.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).