

TM 1333 – BUFFERED LISTERIA ENRICHMENT BROTH BASE (BLE Broth Base) (as per FDA)

INTENDED USE

For selective isolation of *Listeria monocytogenes*.

PRODUCT SUMMARY AND EXPLANATION

Listeria monocytogenes is the only species of the Listeria genus that causes Listeriosis in human, however occasionally L. seeligeri , L. welshimeri and L. ivanovii have been related with human diseases. Microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has show that the principal route of transmission is via the consumption of foodstuffs contaminated with L. monocytogenes.

L. monocytogenes is a well-documented food borne pathogen because of its high morbidity on infection to animals and humans and also due to its psychrotrophic nature exhibiting high tolerance to heat, cold and desiccation. The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water. Listeria species grow over a pH range of 4.4-9.6, and survive in food products with pH levels outside these parameters. Listeria species are microaerophilic, grampositive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C. Food samples are often contaminated with organisms other than Listeria, which makes its isolation difficult. To recover low numbers of L. monocytogenes from food samples, initial enrichment is required. Listeria Enrichment Broth was modified by adding buffering strength thereby making it possible for the medium to be used successfully in conjunction with DNA probe and other methods that are more sensitive than conventional culture procedure. This medium is also recommended by APHA for the selective enrichment of *L. monocytogenes*.

According to FDAs enrichment procedure for isolation of L. monocytogenes from dairy products, the sample to be tested is inoculated in enrichment broth and incubated at 30°C for 4 hours without the selective supplement. After 4 hours the selective supplement is added and further kept for incubation for additional 44 hours at 30°C. After 24 hours and 48 hours the enriched culture is streaked on Oxford Listeria Medium Base and LPM Agar/ Listeria Identification Agar Base, PALCAM and incubated at 35°C for 24-48 hours. Presumptive Listeria colonies are selected and colonies are further purified on Tryptone Soya Yeast Extract Agar. Purified isolates are then subjected to a variety of biochemical tests to confirm the presence of *L. monocytogenes*.

COMPOSITION

Ingredients	Gms / Ltr	
Casein enzymic hydrolysate	17.000	
Papaic digest of soyabean meal	3.000	
Sodium chloride	5.000	
Dipotassium hydrogen phosphate	2.500	
Dextrose	2.500	
Yeast extract	6.000	
Monopotassium phosphate, anhydrous	1.350	
Disodium phosphate, anhydrous	9.600	
Sodium pyruvate	1.000	

PRINCIPLE













Casein enzymic hydrolysate and papaic digest of soyabean meal provide amino acids and other complex nitrogenous substances. Dextrose is the energy source. Sodium pyruvate aids in resuscitation of organisms. The phosphates provide buffering capacity. Sodium chloride maintains the osmotic equilibrium. Yeast extract provides vitamin B complex. The medium is rendered selective due to the inclusion of antimicrobial agents. Cycloheximide inhibits the growth of saprophytic fungi. Nalidixic acid inhibits the growth of gram-negative organisms, whereas acriflavin suppresses growth of gram-positive microorganisms.

INSTRUCTION FOR USE

- Dissolve 23.97 grams in 500 ml distilled water.
- Heat if necessary to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45°C.
- Aseptically add the rehydrated contents of 1 vial of Listeria Selective Supplement II.
- Mix well and dispense as desired.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Appearance of prepared medium : Yellow coloured, clear to slightly opalescent solution with slight precipitate.

pH (at 25°C) : 7.3±0.2

INTERPRETATION

Cultural characteristics observed after incubation with added Listeria Selective Supplement.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
Escherichia coli	25922	>=10³	Inhibited	30°C	24-48 Hours
Listeria innocua	33090	50-100	Good-luxuriant	30°C	24-48 Hours
Listeria monocytogenes	19111	50-100	Good-luxuriant	30°C	24-48 Hours
Listeria monocytogenes	19112	50-100	Good-luxuriant	30°C	24-48 Hours
Listeria monocytogenes	19118	50-100	Good-luxuriant	30°C	24-48 Hours
Staphylococcus aureus	25923	50-100	None-poor	30°C	24-48 Hours
Saccharomyces cerevisiae	9763	50-100	None-poor	30°C	24-48 Hours

PACKAGING:













In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

- 1. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
- 2. Bremer and Osborne, 1995, J. Food Prot., 58:604.
- 3. Patel, Hwang, Beuchat, Doyle and Brackett, 1995, J. Food Prot., 58:244
- 4. Hitchens, 1995, FDA Bacteriological Analytical Manual, 8th Ed. AOAC International, Gaithersburg, Md.
- 5. Murray, Webb and Swann, 1926, J. Pathol. Bacteriol., 29:407.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. *For Lab Use Only

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