



BUFFERED LISTERIA ENRICHMENT BROTH BASE (7675)

Intended Use

Buffered Listeria Enrichment Broth Base (BLEB) is used with supplements for selective enrichment of *Listeria* spp. in foods.

Product Summary and Explanation

Listeria monocytogenes, described first in 1926 by Murray, Webb, and Swann,¹ is an extensive problem in public health and food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women.² Epidemiological evidence from outbreaks of listeriosis has indicated that the principle route of transmission is via consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission include turkey, frankfurters, coleslaw, pasteurized milk, Mexican style cheese, and pate'.⁴

Buffered Listeria Enrichment Broth, a modification of the formula by Lovett et al.⁵ was developed after research concluded that enrichment properties can be improved by increasing the buffering capacity of the medium with the addition of disodium phosphate. BLEB Base is based upon the FDA/BAM recommendations where the medium is supplemented with selective agents after an initial 4 hour, non-selective, pre-enrichment.⁶

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Soybean Meal, and Yeast Extract provides nitrogen, vitamins, and minerals in Buffered Listeria Enrichment Broth Base. Dextrose is the carbohydrate source. Sodium Chloride maintains osmotic balance of the medium. Monopotassium Phosphate, Dipotassium Phosphate, and Disodium Phosphate are the buffering agents. Sodium pyruvate is added aseptically as an oxygen scavenger. Nalidixic acid, Acriflavine and Cycloheximide are added as selective agents after an initial four-hour pre-enrichment. Nalidixic Acid inhibits growth of Gram-negative organisms. Acriflavine inhibits Gram-positive bacteria. Cycloheximide is used to inhibit growth of saprophytic fungi. The delay in adding these agents is intended to facilitate resuscitation, repair, and growth of injured *Listeria* organisms.

Formula / Liter

Enzymatic Digest of Casein	17 g
Enzymatic Digest of Soybean Meal	3 g
Yeast Extract.....	6 g
Dextrose.....	2.5 g
Sodium Chloride	5 g
Monopotassium Phosphate	1.35 g
Dipotassium Phosphate	2.5 g
Disodium Phosphate.....	9.6 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

BLEB Supplement (# 7980)

Acriflavine HCl, 4.5 mg
Nalidixic Acid, 18 mg
Cycloheximide, 22.5 mg

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 47 g of the medium in 1000 mL of purified water.
2. Mix thoroughly.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add 11.1 mL of a 10% filter sterilized solution of sodium pyruvate.
5. After four hours of incubation at 30 ± 2°C, aseptically add 2.5 mL Buffered Listeria Enrichment Supplement (FDA) (#7980) to 225 mL of Buffered Listeria Enrichment Broth Base containing 25 grams of the sample. OR, aseptically add 0.455 mL of a 0.5% aqueous solution of acriflavine, 1.8 mL of a 0.5%

aqueous solution of nalidixic acid, and 1.15 mL of a 1.0% solution of cycloheximide in 40% ethanol to 225 mL of medium containing 25 g or 25 mL of food to be tested.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear, medium amber with none to moderate precipitate.

Expected Cultural Response: Cultural response in Buffered Listeria Enrichment Broth Base, supplemented with selective agents incubated aerobically after 4 hours at 30 ± 2°C, and incubated an additional 20 - 44 hours. Cultures were examined for growth at 18 – 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Growth
<i>Escherichia coli</i> ATCC® 25922	~ 10 ³	Inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	10 - 300	Good growth
<i>Staphylococcus aureus</i> ATCC® 25923	10 - 300	Suppressed at 18 – 24 hours

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Use recommended laboratory procedures for isolating *Listeria* in food samples.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Buffered Listeria Enrichment Broth Base	Code No 7675A	500 g
	7675B	2 kg
	7675C	10 kg
Buffered Listeria Enrichment Supplement (FDA), 5 mL	7980	10 vials/pkg

References

- Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bact. **29**:407-439.
- Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen refrigerated ground beef. J. Food Prot. **57**:969-974.
- Bremer, P.J., and C. M. Osborne. 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58**:604-608.
- Grau, F. H., and P. B. Vanderlinde. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4.7.
- Lovette, J., D. W. Frances, and J. M. Hunt. 1987. *Listeria monocytogenes* In raw milk: detection, incidence and pathogenicity. J. Food Prot. **50**:188-192.
- www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.