

# Listeria Enrichment Broth • Listeria Enrichment Broth, Modified • Buffered Listeria Enrichment Broth Base

## Intended Use

Listeria Enrichment Broth is used to selectively enrich *Listeria* from foods.

Listeria Enrichment Broth, Modified is used for selectively enriching *Listeria* from raw and pasteurized milk according to the International Dairy Federation (IDF).<sup>1</sup>

Buffered Listeria Enrichment Broth Base is used as an enrichment broth for the cultivation of *Listeria* spp. from food according to the U.S Food and Drug Administration (FDA).<sup>2</sup>

## Summary and Explanation

First described in 1926 by Murray, Webb and Swann,<sup>3</sup> *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.<sup>4</sup> The first reported food-borne outbreak of listeriosis was in 1985.<sup>5</sup> Since then, microbiological and epidemiological evidence from both sporadic and epidemic

cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.<sup>6</sup>

Implicated vehicles of transmission include turkey frankfurters,<sup>7</sup> coleslaw, pasteurized milk, Mexican-style cheese, paté, and pickled pork tongue. 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.<sup>8</sup>

*Listeria* species grow over a pH range of 4.4-9.6, and survive in food products with pH levels outside these parameters.<sup>9</sup> *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci, *Bacillus* species, *Escherichia coli*,

## User Quality Control

### Identity Specifications

#### Difco™ Listeria Enrichment Broth or Listeria Enrichment Broth, Modified

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.61% solution, soluble in purified water upon boiling. Solution is light to medium yellowish-amber with a faint green ring at the surface, clear to very slightly opalescent.
Prepared Appearance:	Light yellowish-amber, clear to slightly opalescent.
Reaction of 3.61% Solution at 25°C:	pH 7.3 ± 0.2

#### Difco™ Buffered Listeria Enrichment Broth Base

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.8% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 4.8% Solution at 25°C:	pH 7.3 ± 0.1

### Cultural Response

#### Difco™ Listeria Enrichment Broth

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Enterococcus faecalis</i>	29212	2 × 10 <sup>3</sup> -10 <sup>4</sup>	Inhibition at 18-24 hours; none to poor at 40-48 hours
<i>Escherichia coli</i>	25922	2 × 10 <sup>3</sup> -10 <sup>4</sup>	Inhibition
<i>Listeria monocytogenes</i>	19114	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Saccharomyces cerevisiae</i>	9080	2 × 10 <sup>3</sup> -10 <sup>4</sup>	Inhibition

#### Difco™ Listeria Enrichment Broth, Modified

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Enterococcus faecalis</i>	29212	2 × 10 <sup>3</sup> -10 <sup>4</sup>	Partial inhibition
<i>Escherichia coli</i>	25922	2 × 10 <sup>3</sup> -10 <sup>4</sup>	Marked to complete inhibition
<i>Listeria monocytogenes</i>	19114	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Listeria monocytogenes</i>	19115	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Saccharomyces cerevisiae</i>	9080	2 × 10 <sup>3</sup> -10 <sup>4</sup>	Marked to complete inhibition

#### Difco™ Buffered Listeria Enrichment Broth Base

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 4 hours. After 4 hours of incubation, aseptically add filter-sterilized selective agents according to the label directions. Reincubate at 30 ± 2°C and observe for growth after 48 hours of total incubation.

ORGANISM	ATCC™	INOCULUM CFU	GROWTH
<i>Escherichia coli</i>	25922	10 <sup>3</sup>	Inhibition
<i>Listeria monocytogenes</i>	19114	30-300	Good
<i>Listeria monocytogenes</i>	19115	30-300	Good
<i>Saccharomyces cerevisiae</i>	9080	10 <sup>3</sup>	Inhibition



*Pseudomonas aeruginosa* and *Proteus vulgaris*.<sup>10</sup> Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Listeria Enrichment Broth is based on the formula developed by Lovett et al.<sup>11</sup> in which Tryptic Soy Broth is supplemented with yeast extract for optimum growth of *Listeria*. Listeria Enrichment Broth, Modified is a modification of Listeria Enrichment Broth in which the concentration of one of the selective agents, acriflavine, has been reduced from 15 mg to 10 mg per liter. This modification reflects the lower concentration specified by the IDF for isolation of *L. monocytogenes* from milk and milk products.

Buffered Listeria Enrichment Broth Base is a modification of Listeria Enrichment Broth with added buffering strength. The addition of selective agents is delayed until after four hours of enrichment with this formula.<sup>2</sup>

### Principles of the Procedure

Peptones and yeast extract provide nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Sodium chloride maintains the osmotic balance of the medium. Phosphates provide buffering capacity. Sodium pyruvate aids in resuscitation of stressed organisms. Nalidixic acid inhibits growth of gram-negative organisms. Acriflavine HCl suppresses the growth of gram-positive bacteria. Cycloheximide is incorporated to inhibit saprophytic fungi.

### Formulae

#### Difco™ Listeria Enrichment Broth

Approximate Formula* Per Liter	
Pancreatic Digest of Casein .....	17.0 g
Soytone .....	3.0 g
Dextrose .....	2.5 g
Sodium Chloride .....	5.0 g
Dipotassium Phosphate .....	2.5 g
Yeast Extract .....	6.0 g
Cycloheximide .....	0.05 g
Acriflavine HCl .....	15.0 mg
Nalidixic Acid .....	0.04 g

#### Difco™ Listeria Enrichment Broth, Modified

Approximate Formula* Per Liter	
Pancreatic Digest of Casein .....	17.0 g
Soytone .....	3.0 g
Dextrose .....	2.5 g
Sodium Chloride .....	5.0 g
Dipotassium Phosphate .....	2.5 g
Yeast Extract .....	6.0 g
Cycloheximide .....	0.05 g
Acriflavine HCl .....	10.0 mg
Nalidixic Acid .....	0.04 g

#### Difco™ Buffered Listeria Enrichment Broth Base

Approximate Formula* Per Liter	
Pancreatic Digest of Casein .....	17.0 g
Soytone .....	3.0 g
Dextrose .....	2.5 g
Sodium Chloride .....	5.0 g
Dipotassium Phosphate .....	2.5 g
Disodium Phosphate .....	9.6 g
Monopotassium Phosphate .....	1.35 g
Yeast Extract .....	6.0 g
Sodium Pyruvate .....	1.1 g

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

### Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
  - Difco™ Listeria Enrichment Broth – 36.1 g;
  - Difco™ Listeria Enrichment Broth, Modified – 36.1 g;
  - Difco™ Buffered Listeria Enrichment Broth Base – 48 g.
 Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. For Buffered Listeria Enrichment Broth Base, prepare filter-sterilized solutions of the following agents and add to the base as directed under “Procedure”:
  - 1% (w/v) cycloheximide in 40% (v/v) solution of ethanol in water;
  - 0.5% (w/v) acriflavine HCl in purified water;
  - 0.5% (w/v) nalidixic acid in purified water.
5. Test samples of the finished product for performance using stable, typical control cultures.

### Procedure

#### Listeria Enrichment Broth

For food samples, use Listeria Enrichment Broth in recommended laboratory procedures for isolating *Listeria*.

#### Listeria Enrichment Broth, Modified

For dairy samples, the IDF<sup>1</sup> selective enrichment method is as follows:

1. Add 25 mL of liquid or 25 g of solid test material to 225 mL Listeria Enrichment Broth, Modified and mix or blend thoroughly.
2. Incubate for 48 hours at 30°C.
3. At 48 hours, streak the Listeria Enrichment Broth, Modified culture onto plates of Oxford Medium or PALCAM Medium.
4. Incubate the agar plates at 37°C for 48 ± 2 hours.

#### Buffered Listeria Enrichment Broth Base

For food samples, the FDA<sup>2</sup> selective enrichment method is as follows:

1. Add 25 mL liquid or 25 g solid test material to 225 mL Buffered Listeria Enrichment Broth Base without selective agents and mix or blend thoroughly.
2. Incubate for 4 hours at 30°C.
3. Add 0.455 mL acriflavine HCl solution, 1.8 mL nalidixic acid solution and 1.15 mL cycloheximide solution to 225 mL Buffered Listeria Enrichment Broth Base and continue incubating another 44 hours, for a total of 48 hours, at 30°C.
4. At 24 and 48 hours incubation, streak incubated broth onto both Oxford Medium and LPM Agar (or PALCAM Agar) plates. Incubate Oxford Medium and PALCAM plates at 35°C for 24-48 hours, and LPM plates at 30°C for 24-48 hours.

## Expected Results

1. Examine agar plates for typical *Listeria* colonies.
2. Consult appropriate references for selection of biochemical and/or serological tests for confirmation of *Listeria* spp.<sup>1,2,9,12,13</sup>

## Limitation of the Procedure

*Listeria* Enrichment Broth, Modified is a partially selective medium. Growth of some contaminating strains will be markedly but not totally inhibited.

## References

1. International Dairy Federation. 1995. Milk and milk products - detection of *Listeria monocytogenes*. International IDF Standard No. 143A. International Dairy Federation, Brussels, Belgium.
2. Hitchens. 1995. FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
3. Murray, Webb and Swann. 1926. J. Pathol. Bacteriol. 29:407.
4. Monk, Clavero, Beuchat, Doyle and Brackett. 1994. J. Food Prot. 57:969.
5. Wehr. 1987. J. Assoc. Off. Anal. Chem. 70:769.
6. Bremer and Osborne. 1995. J. Food Prot. 58:604.
7. Grau and Vanderlinde. 1992. J. Food Prot. 55:4.

8. Patel, Hwang, Beuchat, Doyle and Brackett. 1995. J. Food Prot. 58:244.
9. Ryser and Donnelly. 2001. In Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
10. Kramer and Jones. 1969. J. Appl. Bacteriol. 32:381.
11. Lovett, Frances and Hunt. 1987. J. Food Prot. 50:188.
12. Bille, Rocourt and Swaminathan. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
13. Flowers, Andrews, Donnelly and Koenig. 1993. In Marshall (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

## Availability

### Difco™ *Listeria* Enrichment Broth

CCAM COMPF SMD

Cat. No. 222220 Dehydrated – 500 g  
222210 Dehydrated – 10 kg

### Difco™ *Listeria* Enrichment Broth, Modified

Cat. No. 220530 Dehydrated – 500 g  
245152 Dehydrated – 2 kg  
220520 Dehydrated – 10 kg

### Difco™ Buffered *Listeria* Enrichment Broth Base

BAM COMPF

Cat. No. 290720 Dehydrated – 500 g

# Litmus Milk

## Intended Use

Litmus Milk is used for the maintenance of lactic acid bacteria and as a differential medium for determining the action of bacteria on milk.

## Summary and Explanation

Litmus Milk has been used for many years for determining the metabolic activities of microorganisms in milk as an aid to the identification of bacterial species. It is especially useful in species differentiation within the genus *Clostridium*.

This medium is also of value in the maintenance and propagation of lactic bacteria.

## Principles of the Procedure

Skim milk is the substrate that particular species of bacteria attack in different ways to produce various metabolic products. Azolitmin serves as a pH indicator with a color range of pink (below pH 4.5) to purple (in middle of pH range) to blue (above pH 8.3) and also functions as an Eh (oxidation-reduction) indicator.<sup>1</sup>

The action of bacteria on milk can be categorized as follows:

1. No change (no carbohydrate fermentation and no change of litmus indicator).

## User Quality Control

### Identity Specifications

#### BBL™ Litmus Milk

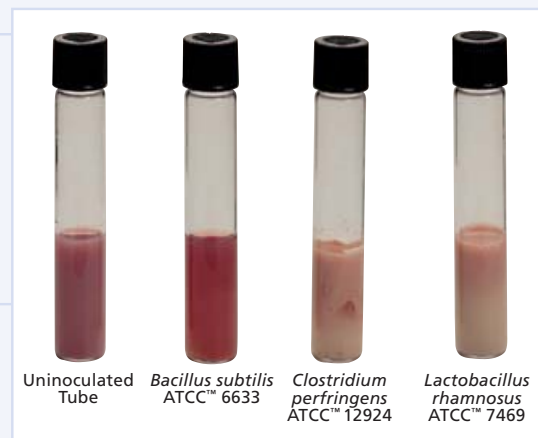
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	10.0% solution, soluble in purified water. Solution is medium, purple gray, opaque.
Prepared Appearance:	Medium, purple gray, opaque.
Reaction of 10.0% Solution at 25°C:	pH 6.8 ± 0.2

### Cultural Response

#### BBL™ Litmus Milk

Prepare the medium per label directions. Inoculate with fresh cultures diluted 1:10 and incubate at 35 ± 2°C for 7 days.

ORGANISM	ATCC™	RESULT
<i>Clostridium perfringens</i>	13124	Stormy fermentation (gas), clot or curd, reduction (white)
<i>Lactobacillus acidophilus</i>	4356	Acid (pink), clot or curd



Uninoculated Tube    *Bacillus subtilis* ATCC™ 6633    *Clostridium perfringens* ATCC™ 12924    *Lactobacillus rhamnosus* ATCC™ 7469