BUFFERED LISTERIA ENRICHMENT BROTH BASE (7675)

Intended Use
Buffered Listeria Enrichment Broth Base (BLEB) is used with supplements for selective enrichment of Listeria spp.

Product Summary and Explanation
Listeria monocytogenes, described first in 1926 by Murray, Webb, and Swann, 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. Epidemiological evidence from outbreaks of listeriosis has indicated that the principle route of transmission is via consumption of foodstuffs contaminated with Listeria monocytogenes. Implicated vehicles of transmission include turkey, frankfurters, coleslaw, pasteurized milk, Mexican style cheese, and pate.

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

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, Acriflavin and Cycloheximide are added as selective agents after an initial four-hour pre-enrichment. Nalidixic Acid inhibits growth of Gram-negative organisms. Acriflavin 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

BLEB Base Supplements/225mL
Acriflavin HCl, 0.5%
Nalidixic Acid, 0.5%
Cycloheximide, 1.0%

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

Directions
1. Dissolve 47 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
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 0.455 mL of a 0.5% aqueous solution of acriflavin, 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 of sample.
**Quality Control Specifications**

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and yellow to tan.

**Prepared Appearance:** Prepared medium is yellow with a green tint.

**Expected Cultural Response:** Cultural response in Buffered Listeria Enrichment Broth Base, supplemented with selective agents after 4 hours of incubation at 30°C, and incubated an additional 20 - 44 hours.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Response</th>
</tr>
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<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC® 25922</td>
<td>inhibited</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em> ATCC® 7644</td>
<td>good growth</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC® 25923</td>
<td>suppressed at 18 – 24 hours</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Buffered Listeria Enrichment Broth Base</th>
<th>Code No.</th>
<th>7675A</th>
<th>500 g</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7675B</td>
<td>2 kg</td>
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<tr>
<td></td>
<td></td>
<td>7675C</td>
<td>10 kg</td>
</tr>
</tbody>
</table>

**References**


**Technical Information**

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.