Fraser Secondary Enrichment HiCynth™ Broth Base

**Intended Use**
Recommended for the isolation, cultivation and enrichment of *Listeria monocytogenes* from food and environmental specimens.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiCynth™ Peptone No.1*</td>
<td>15.000</td>
</tr>
<tr>
<td>HiCynth™ Peptone No.5*</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>20.000</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>3.000</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>12.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.350</td>
</tr>
<tr>
<td>Esculin</td>
<td>1.000</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

* Chemically defined peptones

**Directions**
Suspend 57.85 grams in 990 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45 - 50°C and aseptically add rehydrated contents of 1 vial of Fraser Enrichment Supplement (FD065) or one vial of Fraser Selective Supplement (FD125). Mix thoroughly and dispense into tubes or flasks as desired.

**Principle And Interpretation**
Fraser Secondary Enrichment Broth is a modification of United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) UVM Secondary Enrichment Broth. It is based on the formulation of Fraser and Sperber (2) and found to be remarkably accurate in detecting *Listeria* species in food and environmental samples (5). Fraser Secondary Enrichment Broth is recommended by APHA (6). Fraser Secondary Enrichment Broth Base is formulated so as to provide optimum conditions for the growth of *Listeria*.

Fraser Secondary Enrichment HiCynth™ Broth Base is prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones.
HiCynth™ Peptone No.1, and HiCynth™ Peptone No.5 make the media highly nutritive by providing essential nutrients including carbonaceous and nitrogenous substances. Phosphates maintain the buffering capacity of the medium.

All *Listeria* species exhibit β-glucosidase activity which is evident by the blackening of the media. *Listeria* species hydrolyze esculin (substituted glucoside) to glucose and esculin. The latter combines with ferric ions of ferric ammonium citrate, resulting in the formation of 6-7 dihydroxycoumarin, a black brown complex. Ferric ammonium citrate also enhances the growth of *L. monocytogenes* (1). The high salt tolerance (of sodium chloride) of *Listeria* is used as means to inhibit the growth of Enterococci. Lithium chloride is also used to inhibit Enterococci, which also possess the ability to hydrolyze esculin. Growth of accompanying bacteria is largely inhibited by the addition of Nalidixic acid and Acriflavin hydrochloride (FD).

**Type of specimen**
Food samples and Environmental samples
**Specimen Collection and Handling**
For food samples follow appropriate techniques for handling specimens as per established guidelines (6).
For environmental samples, follow appropriate techniques for sample collection and processing as per guidelines.
After use, contaminated materials must be sterilized by autoclaving before discarding.

**Limitations:**
1. Further biochemical and serological tests must be carried out for complete identification.

**Performance and Evaluation**
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

**Quality Control**

**Appearance**
Cream to yellow homogeneous free flowing powder

**Colour and Clarity of prepared medium**
Basal medium: Yellow coloured, clear solution with slight precipitate. After addition of FD065 or FD125: Fluorescent yellow coloured, clear solution with slight precipitate forms in tubes.

**Reaction**
Reaction of 5.78% w/v aqueous solution at 25°C. pH : 7.2±0.2

**pH**
7.00-7.40

**Cultural Response**
Cultural characteristics observed with added Fraser enrichment supplement (FD065) or Fraser Selective Supplement (FD125) after an incubation at 35-37°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Esculin hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli ATCC 25922</strong></td>
<td>$\geq 10^4$</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td><strong>Enterococcus faecalis ATCC 29212</strong></td>
<td>$\geq 10^4$</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td><strong>Listeria monocytogenes subsp. serovar 1 ATCC 19111</strong></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction, blackening of medium</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes ATCC 19112</strong></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction, blackening of medium</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes ATCC 19117</strong></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction, blackening of medium</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes ATCC 19118</strong></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction, blackening of medium</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus subsp. aureus ATCC 25923</strong></td>
<td>$\geq 10^4$</td>
<td>inhibited</td>
<td></td>
</tr>
</tbody>
</table>

Key : (*) Corresponding WDCM numbers.
Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

Disclaimer:
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