Buffered Peptone Water, Granulated

Buffered Peptone Water, granulated is used as pre-enrichment medium for increasing the recovery of injured *Salmonella* species from foods prior to selective enrichment and isolation. The composition and performance criteria of this medium are as per the applications laid down in ISO 6579-2002.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic digest of casein</td>
<td>10,000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5,000</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate, 12H₂O</td>
<td>9,000</td>
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<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1,500</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 20.07 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Principle And Interpretation**

Microorganisms that are subjected to environmental stresses may become structurally or metabolically damaged or injured. These microorganisms are unable to replicate in selective environments. Therefore these injured organisms must be resuscitated or permitted to repair the damage by incubation in an appropriate, non-selective environment (1). Edel and Kampelmacher (2) noted that sublethal injury to Salmonellae may occur in many food preservation processes. Enriching injured cells in Lactose Broth (pH 6.9) may be further detrimental to their recovery (3). Pre-enrichment in Buffered Peptone Water (M1494I) at 35°C for 18-24 hours results in repair of injured cells (4). The buffering system prevents bacterial damage due to change in the pH of the medium. Recently ISO Committee has also recommended this pre-enrichment medium for the detection of *Enterobacteriaceae* from food stuffs and other materials (5).

Inoculate 10 grams specimen in 50 ml of Buffered Peptone Water (GM1494I/M1494I) and incubate at 35°C for 18 hours. Transfer 10 ml from this medium to 100 ml of Mueller Kauffman Tetraionate Novobiocin Broth Base (GM1496I/M1496I) and Rappaport Vassiliadis Soya Broth (RVS Broth) (GM1491/M1491) and incubate at 43°C for 24-48 hours and then subculture on selective media like XLD Agar, Modified (M031I). Examine the plates for colonies of *Salmonella* species.

**Quality Control**

**Appearance**

Cream to yellow coloured granular medium

**Colour and Clarity of prepared medium**

Light yellow coloured clear solution without any precipitate

**Reaction**

Reaction of 2.0% w/v aqueous solution at 25°C. pH : 7.0±0.2

**pH**

6.80-7.20

**Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours. (Recovery is observed on XLD Agar, M031I)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td></td>
</tr>
</tbody>
</table>
Salmonella Typhi ATCC 6539
50-100 luxuriant >=50%

Salmonella Typhimurium ATCC 14028
50-100 luxuriant >=50%

Escherichia coli ATCC 25922
50-100 fair-good 30-40%

Pseudomonas aeruginosa ATCC 27853
50-100 luxuriant >=50%

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium between 2-8°C. Use before expiry date on the label.

Reference