Peptone, Bacteriological

It contains high tryptophan content used as culture media ingredient in variety of media. It can also be used for commercial production of enzymes, vaccines, antibiotics, steroids and other products.

**Principle And Interpretation**

Peptone, Bacteriological is prepared by enzymatic digestion of selected fresh meat. Being highly nutritious it supports good growth of wide variety of microorganisms and can be used for identification of bacteria by performing various biochemical tests. As peptones confer nutritional benefit, especially at low dilution rates, for the recombinant cell lines it have been recently used as medium additives for the production of a recombinant therapeutic protein in high density perfusion cultures of mammalian cells.

**Quality Control**

**Appearance**
Light yellow to brownish yellow homogenous free flowing powder, having characteristic odour but not putrescent.

**Solubility**
Freely soluble in distilled/purified water, insoluble in alcohol.

**Clarity**
2% w/v aqueous solution remains clear and neutral without any haziness after autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Reaction**
Reaction of 2% w/v aqueous solution at 25°C.

**pH**
6.10-7.10

**Microbial Load:**

**Total aerobic microbial count (cfu/gm)**
By plate method when incubated at 30-35°C for not less than 3 days.
Bacterial Count : <= 2000 CFU/gram

**Total Yeast and mould count (cfu/gm)**
By plate method when incubated at 20-25°C for not less than 5 days.
Yeast & mould Count : <= 100 CFU/gram

**Test for Pathogens**
1. E.coli-Negative in 10 gms of sample
2. Salmonella species-Negative in 10 gms of sample
3. Pseudomonas aeruginosa- Negative in 10 gms of sample
4. Staphylococcus aureus- Negative in 10 gms of sample
5. C.albicans- Negative in 10 gms of sample
6. Clostridia- Negative in 10 gms of sample

**Degree of digestion**

**Nitrite test**
As per method specified in USP 32, NF26 Negative: No development of pink or red colour.

**Microbial Content**
As per method specified in USP 32, NF26 <= Total of 50 microorganisms or clumps in 10 consecutive fields.

**Bacteriological Testing**
Bacteriological tests are carried out as per USP 32, NF26 where respective medium is prepared by using peptone under test.

**Test for fermentable carbohydrate**
Medium : 2% peptone w/phenol red broth w/durhams tube. After inoculation with test culture and incubation for 24 hours at 35-37°C

*Escherichia coli ATCC 25922*
Acid production, (Positive test)

*Streptococcus liquefaciens*
No acid production, (Negative test)
**Production of H2S**
Medium : 1% peptone in water. After inoculation with test culture and incubation for 24 hours at 35-37°C.

- *Enterobacter aerogenes ATCC 13048*
  - Formation of pink colour (Positive test).

- *Escherichia coli ATCC 25922*
  - No formation of pink colour (Negative test).

**Production of Indole**
Medium : 0.1% peptone in water. After inoculation with test culture and incubation for 24 hours at 35-37°C.

- *Escherichia coli ATCC 25922*
  - Appearance of distinct pink to red colour ring (Positive test).

- *Enterobacter aerogenes ATCC 13048*
  - No formation of pink to red coloured ring (Negative test).

**Cultural response**
Cultural response observed after incubation at 35-37°C for 24 hours by using 2% peptone, 0.5% sodium chloride and 1.5% agar in water, pH 7.2-7.4

**Cultural Response**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli ATCC 25922</em></td>
<td>Luxuriant</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa ATCC 27853</em></td>
<td>Luxuriant</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes ATCC 13048</em></td>
<td>Luxuriant</td>
</tr>
<tr>
<td><em>Salmonella Typhi ATCC 6539</em></td>
<td>Luxuriant</td>
</tr>
<tr>
<td><em>Staphylococcus aureus ATCC 25923</em></td>
<td>Luxuriant</td>
</tr>
<tr>
<td><em>Streptomyces albus ATCC 3004</em></td>
<td>luxuriant</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes ATCC 19615</em></td>
<td>luxuriant w/ beta haemolysis (With addition of sterile 5% sheep blood to above medium, after an incubation at 35-37°C for 48 hours).</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae ATCC 19424</em></td>
<td>luxuriant w/ beta haemolysis (With addition of sterile 10% sheep blood to above medium heated to 80-90°C until blood has turned to chocolate brown and incubated in 10% CO2 atmosphere at 35-37°C for 48 hours).</td>
</tr>
</tbody>
</table>

Formation of H2S: The lead acetate test paper shows brownish blackening (lead sulphide).

Formation of Indole: Appearance of distinct pink to red colour ring (Positive test).

No formation of pink colour: No formation of pink to red coloured ring (Negative test).

Production of acetyl methyl carbinol:
Medium : 0.1% peptone and 0.5% of dextrose in water. After inoculation with test culture and incubation for 24 hours at 35-37°C.

- *Escherichia coli ATCC 25922* Formation of pink colour (Positive test).

- *Enterobacter aerogenes ATCC 13048* No formation of pink colour (Negative test).


No formation of acetyl methyl carbinol: No formation of pink colour (Negative test).
Chemical Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nitrogen</td>
<td>$\geq 13.50%$</td>
</tr>
<tr>
<td>Amino Nitrogen</td>
<td>$\geq 3.00%$</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>$\leq 5.0%$</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>$\leq 5.0%$</td>
</tr>
<tr>
<td>Residue on ignition</td>
<td>$\leq 15%$</td>
</tr>
</tbody>
</table>

Storage and Shelf Life

Store below 30°C. Use before expiry date on the label.