Acid Phosphatase Reagent

**Intended Use:**
It is used for the confirmation of *Clostridium perfringens* isolated from water.

**Composition**

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-naphthylphosphate disodium salt</td>
<td>0.200</td>
</tr>
<tr>
<td>Fast Blue B Salt (o-Dianisidine bis(diazotized)Zinc double salt)</td>
<td>0.400</td>
</tr>
<tr>
<td>Acetate buffer</td>
<td>10ml</td>
</tr>
</tbody>
</table>

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

**Directions**

Smear some growth of 24 hours old culture of *Clostridium perfringens* from Blood Agar / Columbia Agar Base / Tryptone Soya Agar (incubated anaerobically at 34-38°C) on the filter paper. Add 2-3 drops of Acid phosphatase Reagent (R096) on to the colonies of filter paper, Observe for appearance of strong purplish colour developed within 3 -4 min which is positive reaction.

**Note:**
1) On standing precipitate may develop, if desired reagent can be filtered to remove precipitate and the filtered reagent can be used for test.
2) Due to inherent composition of product froth may be developed which will not affect the performance criteria of the reagent.

**Principle And Interpretation**

It is an alternative method for the confirmation of *C. perfringens* based upon the expression of acid phosphatase enzyme. Acid Phosphatase catalyzes the hydrolysis of alpha naphthylphosphate, liberating the alpha-naphthol and phosphate, which forms an azo dye with diazonium o-dianisidine (1), that has a strong absorbance at 405 nm. The increase in absorbance is directly proportional to the level of acid phosphatase enzyme.

A positive reaction for acid phosphatase was recorded if a strong purple colour developed within 3-4 min of the reagent being placed on a colony.

**Quality Control**

**Appearance**
Light brown to brown colour solution.

**Clarity**
Hazy solution with precipitate.

Note: Precipitate will not affect the performance criteria of the reagent.

**Cultural Response**
Add 2-3 drops of Acid Phosphatase reagent to a 20-24 hours old culture of the organism under investigation.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Acid Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>strong purple colour developed within 3-4 min</td>
</tr>
<tr>
<td>ATCC 12924</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>strong purple colour developed within 3-4 min</td>
</tr>
<tr>
<td>ATCC 13124</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC</td>
<td>No colour change</td>
</tr>
<tr>
<td>25922</td>
<td></td>
</tr>
</tbody>
</table>
Storage and Shelf Life
Store between 2-8°C in a tightly closed container and away from bright light. Use before expiry date on the label. On opening, product should be stored 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

Disclaimer:
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