



Acid Phosphatase Reagent

R096

Intended use

Acid Phosphatase Reagent is used for the confirmation of *Clostridium perfringens* isolated from water.

Composition**

Ingredients

1-naphthylphosphate disodium salt	0.20 gm
Fast Blue B Salt (o-Dianisidine bis(diazotized)Zinc double salt)	0.40 gm
Acetate buffer	10.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. 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.
2. Add 2-3 drops of Acid phosphatase Reagent (R096) on to the colonies of filter paper.
3. Observe for appearance of strong purplish colour developed within 3-4 min which is positive reaction.

Note: On standing precipitate may develop, if desired reagent can be filtered to remove precipitate and the filtered reagent can be used for test.

4. 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, 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.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective

clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations

1. For further identification confirmatory test is highly recommended.

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** : Light yellow to brown colour solution
- **Clarity** : Hazy solution with precipitate. Note : Precipitate will not affect the performance criteria of the reagent.
- **Cultural Response** :

Organism	Acid Phosphatase
Cultural Response	Add 0.2-0.3 ml of Acid Phosphatase reagent to a 24-48 hours old culture of the organism under investigation.
<i>Clostridium perfringens</i> ATCC 13124 (WDCM00007)	Strong purple colour developed within 3-4 min
<i>Bacillus spizizenii</i> ATCC 6633 (WDCM 00003)	No purple colour developed within 3-4min

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.

Reference

1. Evaluation of acid phosphatase as a confirmation test for *Clostridium perfringens* isolated from water, Sartory DP, Waddock R, Davies CE, Field AM., Lett Appl Microbiol. 2006 Apr;42(4):418-24.
2. I senberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Vol.1 Clinical Microbiology, 11th Edition.
4. Water quality - Enumeration of *Clostridium perfringens*- method using membrane filtration. ISO14189



Storage temperature



Do not use if package is damaged



HiMedia Laboratories Pvt Limited
C-40,21/Y, MIDC, Wagle Ind Area,
Thane(W)-400604,Maharashtra,India

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