



## Acid Phosphatase Reagent

R096

### Intended Use:

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

### Composition\*\*

#### Ingredients

1-naphthylphosphate disodium salt	0.200
Fast Blue B Salt (o-Dianisidine bis(diazotized)Zinc double salt)	0.400
Acetate buffer	10ml

\*\*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 inheritant 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.

Organism	Acid Phosphatase
<i>Clostridium perfringens</i> ATCC 12924	strong purple colour developed within 3-4 min
<i>Clostridium perfringens</i> ATCC 13124	strong purple colour developed within 3-4 min
<i>Escherichia coli</i> ATCC 25922	No colour change

## 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

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

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### Disclaimer :

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