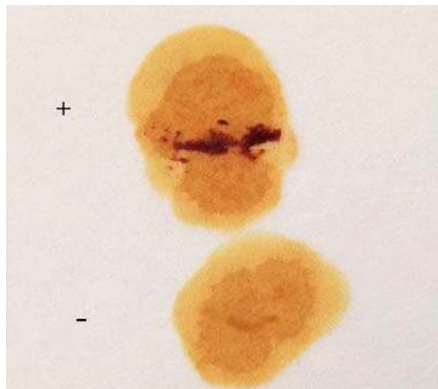


ACID PHOSPHATASE REAGENTS



Acid Phosphatase Test: *C. perfringens* (+),
C. bifermentans (-)

1 - INTENDED USE

For the confirmation test of *Clostridium perfringens* colonies according to ISO 14189 and ISO 15213-2.

2 - COMPOSITION – PACK CONTENT

Acid phosphatase reagent -vial A

1- Naftyl phosphate monosodium salt	0.08 g
Acetic acid	0.0012 mL
Sodium acetate	0.0016 g
Purified water	4 mL

Acid phosphatase reagent -vial B

Fast Blue B	0.16 g
Acetic acid	0.0012 mL
Sodium acetate	0.0016 g
Purified water	4 mL

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Acid phosphatase catalyses the hydrolysis of the naphthyl phosphate, releasing alpha-naphthol and phosphate and forming an azo dye with the Fast Blue B which results in the development of a purple colour.

The acid phosphatase test is indicated as confirmation test of *C. perfringens* colonies grown on TSC Agar and sub-cultured on blood agar, according to ISO 14189¹ and ISO 15213-2².

4 – CHARACTERISTICS OF REAGENTS

Acid Phosphatase Reagent- Vial A: 2% Naftyl phosphate monosodium salt in acetate buffer, clear colourless solution

Acid Phosphatase Reagent -Vial B: 4% Fast Blue B in acetate buffer, cloudy brown-orange solution with possible precipitates (formulations in accordance with the concentrations reported in ISO 14189 and 15213-2)

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Acid Phosphatase Reagents	Liquid reagents	192010	4+4 mL (80 tests)

Acid Phosphatase Reagent- Vial A: vial with dropper cap containing 4 mL of solution.

Acid Phosphatase Reagent- Vial B: vial with dropper cap containing 4 mL of solution.

Both reagents are ready-to-use for performing the acid phosphatase test.

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Microbiology loops, filter paper, incubator, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

The test is performed on microbial colonies grown on Columbia Blood Agar or Blood Agar plates or other suitable medium according to ISO 14189 and ISO 15213-2.

8 - TEST PROCEDURE

- Obtain the specimen from primary isolation culture streaked on Blood Agar or Columbia Blood Agar or Tryptic Soy Agar plates.
- Incubate at 36 ± 2 °C for 21 ± 3 hours in anaerobiosis.
- Streak some colonies grown on the incubated medium as described above on filter paper and add 1-2 drops of Acid Phosphatase Reagent- Vial A and immediately afterwards 1-2 drops of Acid Phosphatase Reagent- Vial B.
- Wait 3-4 minutes for reading the results.

9 - READING AND INTERPRETATION

Examine the reaction area for the appearance of a purple colour.

Positive test: the development of a purple colour after 3-4 minutes from the addition of the reagent indicates the positivity to the test of the acid phosphatase.

Negative test: if there is no purple colour development, the test is considered negative.

Clostridium perfringens develops black or gray to brown-yellow colonies on TSC Agar, positive for acid phosphatase testing.

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.

Positive control

C. perfringens ATCC 13124 purple colour development

C. perfringens NCTC 8238 purple colour development

Negative control

C. bifermentans NCTC 506 no purple colour development



B. subtilis ATCC 6633 no purple colour development
 ATCC is a trademark of American Type Culture Collection; NCTC: National Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of Acid Phosphatase Reagents is tested with positive and negative strains. Phosphatase positive strains (purplish colour development within 3-4 minutes): *C. perfringens* ATCC 13124, *C. perfringens* ATCC 12916, *C. perfringens* NCTC 13170
 Phosphatase negative strains (non colour change within 4 minutes): *C. bifermentans* NCTC 506, *B. subtilis* ATCC 6633.

12 - LIMITATIONS OF THE METHOD

- Readings over 4 minutes may give dubious or confusing results.

13 - PRECAUTIONS AND WARNINGS

- This product is for laboratory control, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is classified as dangerous according to the current European legislation. Consult the Safety Data Sheet before use.
- The reagents are not sterile.
- Apply good laboratory practice guidelines when performing the test.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as the reagents, the culture media or the microbial strains.
- Sterilize all biohazard waste before disposal. Dispose the used strips and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.









14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original pack at 2-8°C away from direct light. If properly stored, the product may be used up to the expiration date. Do not use the product beyond this date. Opened container can be used up to the expiration date. Repeated openings of the container do not affect the performances of the product. The presence of precipitates in the reagent Vial B does not affect performance; however, it can be filtered before use on filter paper.

15 - REFERENCES

- ISO 14189:2013. Water quality-Enumeration of *Clostridium perfringens*-Method using membrane filtration.
- ISO 15213-2:2023 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. —Part 2: Enumeration of *Clostridium perfringens* by colony-count technique

TABLE OF APPLICABLE SYMBOLS

 or REF Catalogue number	 Batch code	 Manufacturer	 Keep away from direct light
 Temperature limitation	 Content sufficient for <n> tests	 Consult Instructions for Use	 Use by

REVISION HISTORY

Version	Description of changes	Date
Revision 3	Updated layout and content	2023/03
Revision 4	Normative update	2024/03
Revision 5	Entering the number of tests	2025/06

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

