Instructions for use
BIOK382-CPAT_NO_(EN)_V02
20/11/2024

Multiscreen AgELISA Alpha toxin – *Clostridium perfringens*

Reference: BIO K 382

ELISA kit for detection of *Clostridium perfringens* and Alpha toxin
Biwell, sandwich

In vitro and strictly veterinary use

Sample / Dilution	All species
Culture supernatants / 1X	✓
Biological samples / 2X	✓

Presentation

Product reference	BIO K 382/2
Format	2 plates, strip of 8 wells
Reactions	48 tests

Composition of the kit

Provided material		BIO K 382/2
Microplate	Microplate	2
Washing solution	Washing solution (20X)	1 X 100 mL
Dilution solution	Dilution solution (5X)	1 X 50 mL
Conjugate anti-Alpha	Conjugate anti-Alpha toxin (1X - red)	1 X 12 mL
Conjugate anti-*Clos.perf.*	Conjugate anti-*Clos. Perf.* (1X - green)	1 X 12 mL
CTL POS	Positive control (1X)	1 X 4 mL
TMB solution	Single component TMB (1X)	1 X 25 mL
Stop solution	Stop solution (1X)	1 X 15 mL

Revision history

Date	Version	Modifications
20/11/2024	V02	Layout and simplification of the entire manual.

Note : minor changes to typography, grammar and formatting are not included in the revision history

A. Introduction

Enterotoxaemia is a fatal enteric disease that affects all species of domestic animals and is attributable to a toxigenic type of *Clostridium perfringens*. The latter is an anaerobic, strongly gram-positive bacterium that has the ability to form heat-resistant endospores. This bacterium is grouped into five types (types A, B, C, D and E) according to the four major lethal toxins, Alpha, Beta, Epsilon, and iota (α, β, ε, ι) produced.

Clostridium perfringens has been shown to be a cause of human diseases such as gas gangrene (clostridial myonecrosis), food poisoning, necrotising enterocolitis of infants, and enteritis necroticans (pigbel). It is also the causative agent of lamb dysentery, ovine enterotoxaemia (struck) and pulpy kidney disease of sheep, and other enterotoxemic diseases of lambs and calves. Large amounts of toxin in addition to large numbers of *Clostridium perfringens* cells can usually be detected in the intestinal fluid of diseased or dead animals.

As *Clostridium perfringens* is a natural commensal of human and animal intestines, identifying the bacterium is not enough. Toxinotyping and quantifying of the isolated strains are essential.

The Bio-X multiscreen alpha toxin – *Clostridium perfringens* Elisa Kit can detect the Alpha, toxin of *Clostridium perfringens* and reveal the multiplication of the bacterium.

The kit works with culture fluids as well as biological probes such as liquid intestinal contents.

B. Test principle

Rows A and E are sensitized by specific monoclonal antibody produced against α-toxin of *Clostridium perfringens* and rows C and G by monoclonal antibody specific for a structural protein of this bacterium. These antibodies allow specifically the capture of the toxin or bacteria that may be present in the samples (intestinal fluid).

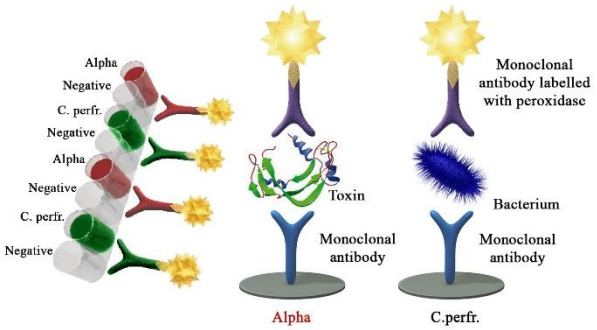
Rows B, D, F, H are containing non-specific antibodies. These control rows allow the differentiation between specific immunological reaction and non-specific bindings so as to eliminate false positives. All samples except culture supernatants are diluted in dilution solution and incubated on the microplate for 1 hour at 21±3°C.

After this first incubation step, the plate is washed, then conjugates (peroxidase-labelled anti-α-toxin monoclonal or *C. perfringens* monoclonal) are added to the wells. The plate is then re-incubated for 1 hour at 21±3°C. after this second incubation step, the plate is washed again and the chromogen (tetramethylbenzidine TMB) is added. This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and not being carcinogenic.

If α-toxins and/or *C. perfringens* are present in the tested samples, conjugates remain bound to the corresponding microwells, and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the content of the toxin or pathogenic agent of the sample. The enzymatic reaction can be stopped by acidification and the resulting optical density at 450 nm can be read using a photometer. The signals read for the negative control microwells are subtracted from the corresponding positive microwells.

A positive control is provided with the kit so as to validate the test results.

Toxinotype	Alpha	Beta	Epsilon	Iota
A	++	-	-	-
B	+	++	+	-
C	+	++	-	-
D	+	-	++	-
E	+	-	-	++



C. Additional material and required equipment (not provided)

- Distilled/demineralized water
- Graduated mono- or multichannel pipettes (2-20 µL, 20-200 µL et 100-1000 µL range) and single-use tips
- Microplate reader (450nm filter)
- Microplate washer (optional)
- Dilution microplate (optional)
- Incubator at 21±3°C
- Standard laboratory equipment: graduated cylinder, tube rack, lid, …

D. Precautions for use

- The reagents must be kept between +2 et +8°C.
- Unused strips must be stored with the desiccant in the hermetically sealed aluminum envelope.
- Do not use reagents beyond shelf-life date.
- Do not use reagents from other kits.
- Make sure to use distilled/demineralized water.
- The stopping solution contains 1 M phosphoric acid. Handle it carefully.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- Keep the TMB solution away from light.

E. Preparation of solutions

- The solutions are to be prepared extemporaneously.
- The washing solution must be diluted 20-fold in distilled/demineralized water. The cold solution crystallizes spontaneously. Bring the vial to 21±3°C to make sure that all crystals have disappeared; mix the solution well and withdraw the necessary volume.
- The dilution solution must be diluted 5-fold in distilled/demineralized water. The dilution solution is colored in yellow, it is used for the dilution of biological samples.
- The positive control is ready to use.
- The conjugates are ready to use. Each valence has a corresponding color: anti-toxin alpha (red), and anti-*Clostridium perfringens* (green).
- The stop solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Preparation of samples

- **Biological samples** (intestinal contents) must be diluted **2-fold** in the dilution solution.

N.B : The consistency of the sample must be homogeneous. If homogenization is difficult, add glass beads to the container and break up the stool by mixing. Do not centrifuge.

- **Culture supernatants** are used undiluted.

N.B : For optimum detection of Alpha toxin, we recommend 8h culture at 37°C in TGY medium under anaerobic conditions (e.g. in a 10 ml tube of culture medium, tightly closed, without agitation). After 8h incubation, freeze the culture until use. The signal recorded in the C.

perfringens wells (C-D and G-H) will allow to objectivize the presence of the bacteria and its growth.

Composition of TGY :

- Trypticase (casein tryptic peptone) 30g
- Yeast extract 20g
- Glucose 1g
- L-cysteine 1g

Dissolve trypticase and yeast extract in 950 mL of water and autoclave. Dissolve glucose and L-cysteine in 50 mL of water and sterilize by filtration. Mix the two solutions when the first one is cooled.

G. Procedure

- Bring all the reagents to **21±3°C** before use.
- Carefully read through the previous points.

***N.B.** : To avoid differences in incubation time between samples of a large series, sample dilutions and reference dilutions can be prepared in a dilution microplate before transfer (100 µL) into the test microplate using a multi-channel pipette.*

1. Distribute **100 µL per well** of **diluted** biological samples, the positive control of the kit, **ready to use,** and the **undiluted** culture supernatants. A sample or positive control **per column** (e.g. : sample n°1 in the wells A1 to D1; sample n°2 in wells E1 to H1). Cover and incubate the plate **at 21 ± 3°C** during **60 ± 5 min**.

2. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL** of washing solution per well. Avoid the formation of bubbles in the well between each wash.

3. Add **100 µL ready to use conjugate** per well.

Conjugate	Distribution
Anti-Alpha toxin conjugate (1X – red)	Line A-B, E-F
Anti-Clos. Perf. conjugate (1X – green)	Line C-D, G-H

   Cover and incubate the plate **at 21 ± 3°C** during **60 ± 5 min**.

4. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL** of washing solution per well. Avoid the formation of bubbles in the well between each wash.

5. Distribute **100 µL** of TMB solution per well. Incubate at **21 ± 3°C** for **10 ± 1 min** away from the light, without covering.

6. Distribute the **stop solution** at rate of **50 µL** per well. The colour changes from blue to yellow.

7. Record the optical densities using a plate spectrophotometer with a **450 nm** filter **within 5 minutes** after adding the stop solution.

H. Validation of results

The test can only be **validated** if the positive control antigen yields a difference in the optical density at 10 minutes that is greater than the value given on the QC data sheet.

I. Interpretation of results

Calculate the net optical density of each sample by subtracting from the reading for each sample well the optical density of the corresponding negative control.
Proceed the same way for the positive control.

Divide the signal read for each sample well by the corresponding positive control signal and multiply this result by 100 to express it as a percentage.

$$\text{Val (ue)} = \frac{\text{Delta OD sample} \times 100}{\text{Delta OD positive}}$$

Using the first table in the quality control procedure, determine each sample's status (positive, negative).

Get the interpretation of your results quickly and easily using **AnalysiScreen**, our free online platform, available on our website : https://www.biox.com

AnalysiScreen™ is the new module for reading and interpreting all types of Monoscreen™ and Multiscreen™ ELISA plates. **AnalysiScreen™** is :

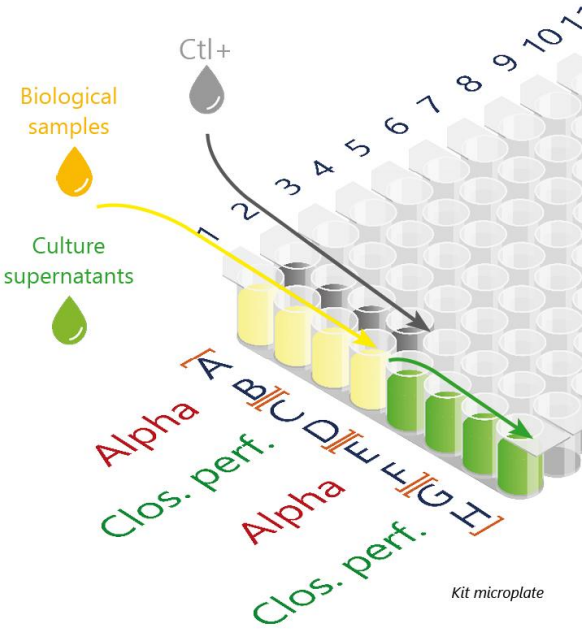
- Free
- Accessible online via our website: https://www.biox.com
- Updated in real time
- Compatible with all Bio-X Diagnostics plate designs
- Very easy to use

SCAN ME

Notes*

1 Dilution of biological samples 1/2
Culture supernatants 1/1
Positive control (Ctl+) 1/1

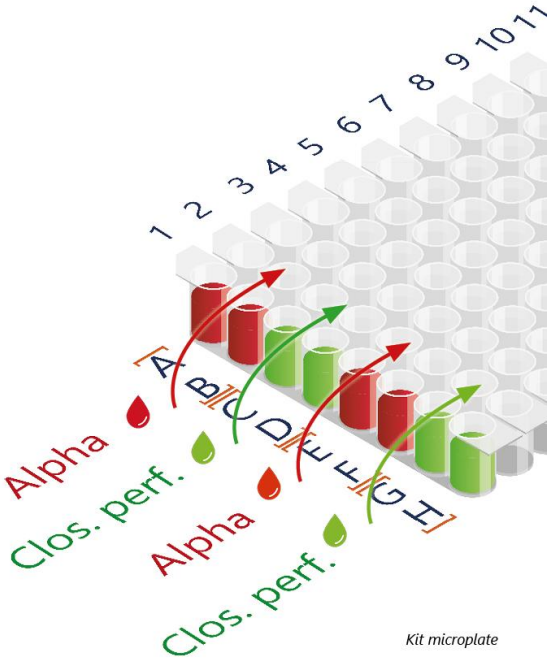
Room Temp. — 60 min → 3X Wash



Kit microplate

2 Add 100 µL of conjugate

Room Temp. — 60 min → 3X Wash



Kit microplate

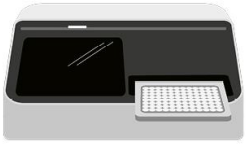
3 Add 100 µL of TMB

Room Temp. — 10 min — Obscurity

4 Add 50 µL of stop solution

5 Record optical densities

450 nm



* Notes do not replace the instructions for use of which they are a synthesis.

Contact us
support.immuno@biox.com
www.biox.com +32 (0) 84 32 23 77
Bio-X Diagnostics
38, rue de la Calestienne
5580 Rochefort (Belgium)