



MULTISCREEN[®] Ag ELISA

Instructions for use
BIOK290-Enterotoxemia_NO_(EN)_V01
10/06/2024

Multiscreen AgELISA Enterotoxemia

Reference : BIO K 290

ELISA kit for detection of *Clostridium perfringens* and Alpha, Beta and Epsilon toxins

Biwell, sandwich

In vitro and strictly veterinary use



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

Presentation

Product reference	BIO K 290/2
Format	2 plates, strip of 8 wells
Reactions	24 tests

Composition of the kit

Provided material		BIO K 290/2
Microplate	Microplate	2
Washing solution	Washing solution (20X)	1 X 100 mL
Dilution solution	Colored dilution solution (5X)	1 X 50 mL
Conjugate anti-Alpha	Anti-Alpha-toxin conjugate (1X – red)	1 X 6 mL
Conjugate anti-Beta	Anti-Beta-toxin conjugate (1X – yellow)	1 X 6 mL
Conjugate anti-Epsilon	Anti-Epsilon-toxin conjugate (1X – blue)	1 X 6 mL
Conjugate	Anti-Clos. Perf. conjugate (1X – green)	1 X 6 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
10/06/2024	V01	Creation

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 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 enterotoxaemic 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 the diseased or dead animals.

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

The Bio-X Enterotoxaemia Elisa Kit can detect the Alpha, Beta and Epsilon toxins of *Clostridium perfringens* and reveal the multiplication of the bacterium.

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

B. Test principle

Specific monoclonal antibodies produced against Alpha, Beta and Epsilon-toxins of *Clostridium perfringens* and a monoclonal antibody specific for a structural protein of this bacterium have been immobilized on alternate rows of 8 x 12 well - microtitre plates. These antibodies allow specifically the capture of the corresponding toxins or bacteria that may be present in the samples (intestinal fluid, culture supernatants, etc.). Rows A, C, E, G have been sensitised respectively with these antibodies and rows B, D, F and H are coated with aspecific antibodies as controls.

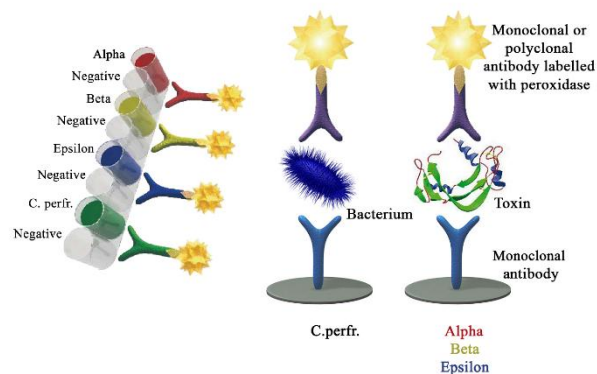
All samples except culture supernatants are diluted in dilution solution and incubated on the microplate for 1 hour at 21°C +/- 3°C.

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

If specific toxins 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 specific pathogen titre in the sample and the concentration of toxins present. 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.

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

Toxinotypes	Alpha	Theta	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).
- 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 positive control is ready to use.
- The conjugates are ready to use.
- 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 to perform an 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 (G-H) will reveal the presence and growth of the

bacteria. An incubation period of 8h at 37°C is also suitable for the production of Beta and Epsilon toxins.

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 can be prepared and control and culture supernatants can be distributed in a dilution microplate before transfer (100 µL) into the test microplate using a multi-channel pipette.

- 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 H1). Cover and incubate the plate at **21 ± 3°C** during **60 ± 5 min**.
- 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.
- Add **100 µL ready to use conjugate** per well.

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

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

- 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.
- Distribute **100 µL** of TMB solution per well. Incubate at **21 ± 3°C** for **10 ± 1 min** away from the light, without covering.
- Distribute the **stop solution** at rate of **50 µL** per well. The colour changes from blue to yellow.
- 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 difference between optical density readings (OD) of the odd and even line of the positive control is greater than the value on the QC data sheet included in the kit for each of the toxins and *Clostridium perfringens*.

- + Control / Alpha-toxin : OD_{line A} - OD_{line B} > QC
- + Control / Beta-toxin : OD_{line C} - OD_{line D} > QC
- + Control / Epsilon-toxin : OD_{line E} - OD_{line F} > QC
- + Control / Clos. perf. : OD_{line G} - OD_{line H} > QC

I. Interpretation of results

Calculate for each sample its coefficient (S/P %) for each of the toxins and *Clostridium perfringens* using the following formula :

$$S/P_{\text{Alpha}} (\%) = \frac{\text{OD sample line A} - \text{OD sample line B}}{\text{OD positive control line A} - \text{OD positive control line B}} * 100$$

$$S/P_{\text{Beta}} (\%) = \frac{\text{OD sample line C} - \text{OD sample line D}}{\text{OD positive control line C} - \text{OD positive control line D}} * 100$$

$$S/P_{\text{Epsilon}} (\%) = \frac{\text{OD sample line E} - \text{OD sample line F}}{\text{OD positive control line E} - \text{OD positive control line F}} * 100$$

$$S/P_{\text{Clos. p.}} (\%) = \frac{\text{OD sample line G} - \text{OD sample line H}}{\text{OD positive control line G} - \text{OD positive control line H}} * 100$$

	Results	Status
Sample	Alpha S/P % < x* %	Negative
	Alpha S/P % ≥ x* %	Positive
	Beta S/P % < x* %	Negative
	Beta S/P % ≥ x* %	Positive
	Epsilon S/P % < x* %	Negative
	Epsilon S/P % ≥ x* %	Positive
	Clos. perf. S/P % < x* %	Negative
	Clos. perf. S/P % ≥ x* %	Positive

*Determine samples' status for each of the toxins and *Clostridium perfringens* using the table in the quality procedure (QC) included in the kit.

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

ANALYSISCREEN

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

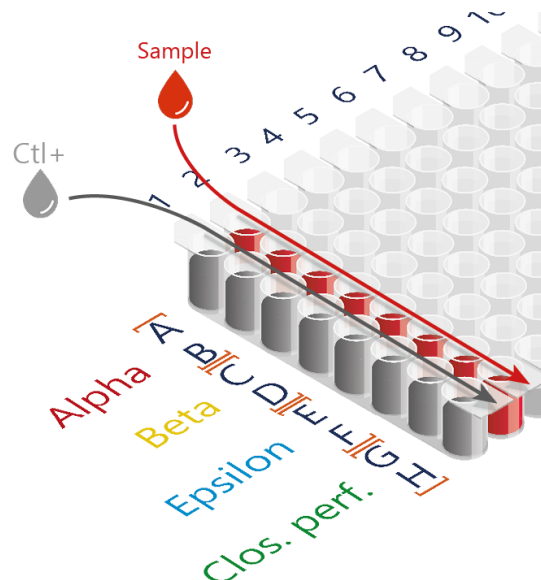
- Free
- Accessible online via our website: <https://www.biox.com>
- Updated in real time
- Compatible with all Bio-X Diagnostics plate designs
- Very easy to use



SCAN ME

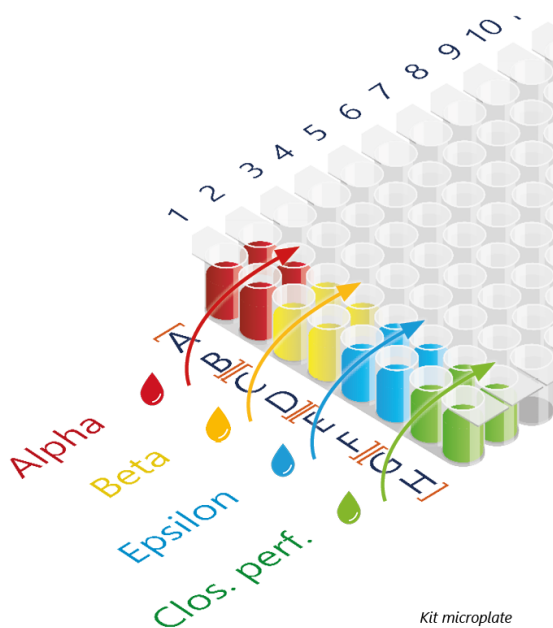
Notes*

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



Kit microplate

- 2 Add 100 μ L of conjugate



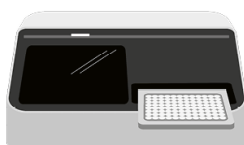
Kit microplate

- 3 Add 100 μ L of TMB



- 4 Add 50 μ L of stop solution

- 5 Record optical densities



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