MULTISCREEN Ag ELISA

Instructions for use
BIOK270-Enteretoxemia_NO_(EN)_V03
04/07/2022

Multiscreen AgELISA *Enterotoxemia*

Reference : BIO K 270

ELISA kit for detection of *Clostridium perfringens* and Theta, Beta and Epsilon toxins
Biwell, sandwich

In vitro and strictly veterinary use

Sample	All species
Culture supernatants	✓
Biological fluids	✓

Presentation

Product reference	BIO K 270/2
Format	2 plates, strip of 8 wells
Reactions	24 tests

Composition of the kit

Provided material	BIO K 270/2
Microplate	2
Washing solution (20X)	1 X 100 mL
Colored dilution solution (5X)	1 X 50 mL
Theta-toxin conjugate (1X – red)	1 X 6 mL
Beta-toxin conjugate (1X – yellow)	1 X 6 mL
Epsilon-toxin conjugate (1X – blue)	1 X 6 mL
Clos. Perf. conjugate (1X – green)	1 X 6 mL
Positive control (1X)	1 X 4 mL
Single component TMB (1X)	1 X 25 mL
Stop solution (1X)	1 X 15 mL

Revision history

Date	Version	Modifications
04/07/2022	V03	Layout and simplification of the entire leaflet Replacement of the designation “alpha-toxin” by “theta-toxin”

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 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 and pericardial- or peritoneal fluid.

**Recent mass spectrometry analyses have allowed us to demonstrate that the monoclonal antibody used in this kit is not specific for alpha toxin but for theta toxin and a protease.*

As theta toxin is almost always associated with alpha toxin, the purpose of this diagnostic kit for enterotoxemia is not affected by this analytical advance. Moreover, theta toxin is less sensitive to the effect of proteases than alpha toxin and is therefore a more sensitive marker of Clostridium perfringens.

B. Test principle

Specific monoclonal and polyclonal antibodies produced against Thêta, 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, body fluids, etc.). Rows A, C, E, G have been sensitised 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 buffer 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. 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.

Control antigen is provided with the kit so as to validate the test results

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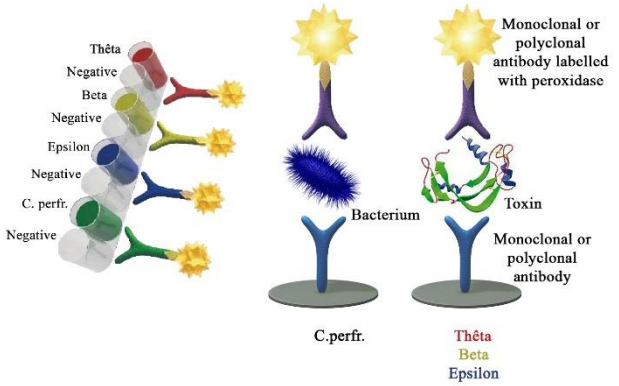
Toxinotypes	Alpha	Theta	Beta	Epsilon	Iota
A	++	++	-	-	-
B	+	++	++	+	-
C	+	++	++	-	-
D	+	++	-	++	-
E	+	++	-	-	++



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
- Incubator at 37±2°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 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

- The positive control is ready to use.
- Biological samples (intestinal contents, cavity fluids, ...) **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 shaking vigorously. Do not centrifuge.

- Culture supernatants are used **undiluted**.

N.B : The best results have been obtained by using liquid TGY under anaerobic conditions (in a tube without shaking) at 37°C. For Epsilon- and Beta-toxin the samples may be cultured for 8 hours or preferably overnight. When no information about the strain is available, it is preferable to incubate for 8 hours at 37°C without shaking.

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 and a sample or positive control per column (e.g. : sample n°1 in the wells A1 to H1). Cover with a lid 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
Theta-toxin conjugate (1X – red)	Line A-B
Beta-toxin conjugate (1X – yellow)	Line C-D
Epsilon-toxin conjugate (1X – blue)	Line E-F
Clos. Perf. conjugate (1X – green)	Line G-H

Cover with a lid 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. Incuber à 21 ± 3°C pendant 10 ± 1 min à l'abri de la lumière, sans couvrir.

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 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 / theta-toxin : $OD_{\text{line A}} - OD_{\text{line B}} > QC$

+ Control / beta-toxin : $OD_{\text{line C}} - OD_{\text{line D}} > QC$

+ Control / epsilon-toxin : $OD_{\text{line E}} - OD_{\text{line F}} > QC$

+ Control / *Clos. perf.*-toxin : $OD_{\text{line G}} - OD_{\text{line H}} > QC$

I. Interpretation of results

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

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

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

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

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

	Results	Status
Sample	Theta S/P % < x* %	Negative
	Theta 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 :

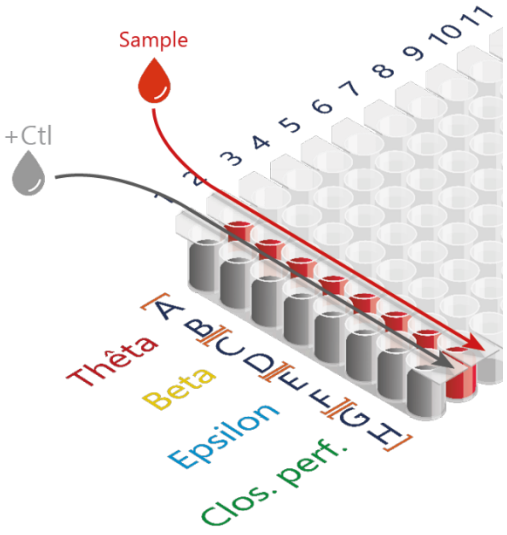
- Free
- Accessible online via our website: https://www.biox.com
- Updated in real time
- Compatible with all Bio-X Diagnostics plate designs
- Very easy to use

SCAN ME

Notes*

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

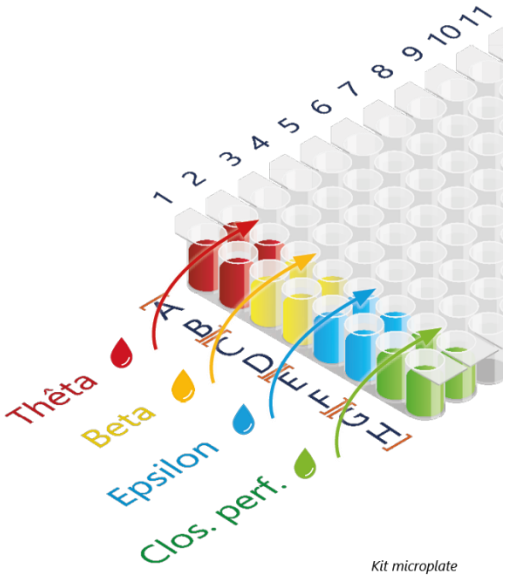
Room Temp. — 60 min → 3X Wash



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
info@biox.com
+32 (0) 84 32 23 77
www.biox.com