MONOSCREEN Ag ELISA

Notice d'utilisation
BIOK268-CPET_NO_(EN)_V02
10/06/2024

Monoscreen AgELISA *Clostridium perfringens* Epsilon toxin

Reference : BIO K 268

ELISA kit for antigenic detection of *Clostridium perfringens* Epsilon toxin
Biwell, sandwich test

For veterinary *in vitro* use only

Sample / dilution	All species
Culture supernatants / 1X	✓
Biological fluids / 2X	✓

Presentation

Product reference	BIO K 268/2
Format	2 plates, strips of 8 wells
Reactions	96 tests

Kit composition

Provided material		BIO K 268/2
Microplate	Microplates	2
Washing solution	Washing solution (20X)	1 x 100 mL
Dilution solution	Colored dilution solution (5X)	1 x 50 mL
TMB solution	Single component TMB (1X)	1 x 25 mL
Stop solution	Stopping solution (1X)	1 x 15 mL
Conjugate	Conjugate (1X)	1 x 25 mL
CTL POS	Positive control (1X)	1 x 4 mL

Revision history

Date	Version	Modifications
10/06/2024	V02	Layout and simplification of the entire manual

Note: minor typographical, grammar and formatting changes 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, necrotizing 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.

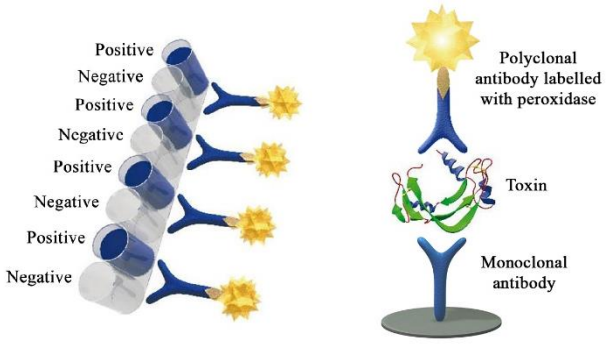
The kit works with culture supernatants as well as biological probes such as liquid intestinal contents and pericardial- or peritoneal fluid.

B. Test principle

The test uses 96-well microtitration plates sensitized by specific monoclonal antibodies for the Epsilon-toxin. These antibodies allow a specific capture of the corresponding antigen which is present in the samples. Rows A, C, E, G have been sensitized with these antibodies and rows B, D, F, H are containing non-specific antibodies. These control rows allow the differentiation between specific immunological reaction and non-specific bindings. Biological samples (for example: contents of the small intestine, peritoneal fluid....) are diluted in dilution solution and incubated on the microplate for 1 hour at 21°C +/- 3°C. Culture supernatants are used without dilution.

After this first incubation step, the plate is washed and incubated for 1 hour with the conjugate - a peroxidase labelled anti-Epsilon-toxin specific polyclonal antibody. After this second incubation, the plate is washed again, and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic.

If Epsilon-toxin is present in the tested samples, the conjugate remains bound to the corresponding microwells, and the enzyme catalyzes the transformation of the colorless chromogen into a pigmented compound. The intensity of the resulting blue color is proportionate to the titre of Epsilon-toxin in the sample. Enzymatic reaction can be stopped by acidification and resulting optical density at 450 nm can be recorded using a photometer. The signals recorded for the negative control microwells are subtracted from the corresponding positive microwells.
There is a positive control supplied with the kit.



C. Material required but not provided

- Distilled/demineralized water.
- Dilution microplates.
- Graduated mono or multichannel pipettes (2-20 µL, 20-200 µL and 10-1000 µL range) and single-use tips.
- Microplate reader (450nm filter).
- Microplate washer.
- Incubator at 21±3°C.
- Standard laboratory equipment: graduated cylinder, tube rack, lid,...

D. Warnings and precautions of use

- The reagents must be kept between +2 and +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 1M 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 the 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 is to be diluted 5 times in distilled/demineralized water. The dilution is colored in yellow. It is used for dilution of biological fluids.
- The positive control is ready to use.
- The conjugate is ready to use.
- The stopping solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Preparation of the samples

- Dilute the **biological fluids** at **½** in the dilution solution. Discards eventual gruds by natural decantation for about 10 minutes. Do not centrifuge the suspensions.

- **Culture supernatants** are used undiluted.

  The best results are obtained by using liquid TGY under anaerobic conditions at 37°C. The samples may be cultured for 8 hours or overnight.

  Composition of TGY:

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

  Dissolve trypticase and yest 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 at 21±3°C.

G. Procedure

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

1. Distribute the **diluted biological samples**, the **non-diluted supernatants**, and the **non-diluted positive control** at a rate of **100 µL** per well.
   Cover with a lid and incubate at **21±3°C** for **60±5min**.

2. Remove the content of the microplate. **Wash the microplate 3 times** with **300µL of washing solution per well.** Avoid the formation of bubbles in the wells between each wash.
3. Distribute the **conjugate** at a rate of **100µL** per well. Cover with a lid and incubate at **21±3°C** for **60±5min**.
4. Remove the content of the microplate. **Wash the microplate 3 times** with **300µL of washing solution per well.** Avoid the formation of bubbles in the wells between each wash.
5. Distribute **100µL** of **TMB solution** per well. Incubate at **21±3°C** for **10±1min** away from the light, without covering.
6. Distribute the **stopping solution** at a rate of **50µL per well**. Color changes from blue to yellow.
7. Record optical densities using a plate spectrophotometer with a **450nm filter** within **5 minutes** after adding the stopping solution.

H. Validation of results

The test can only be **validated** if:

- The **positive control** 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 (OD) of each sample by subtracting from the reading for each sample well (A, C, E, G) the optical density of the corresponding negative control (B, D, F, H).

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.

$$\text{Val(ue)} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

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

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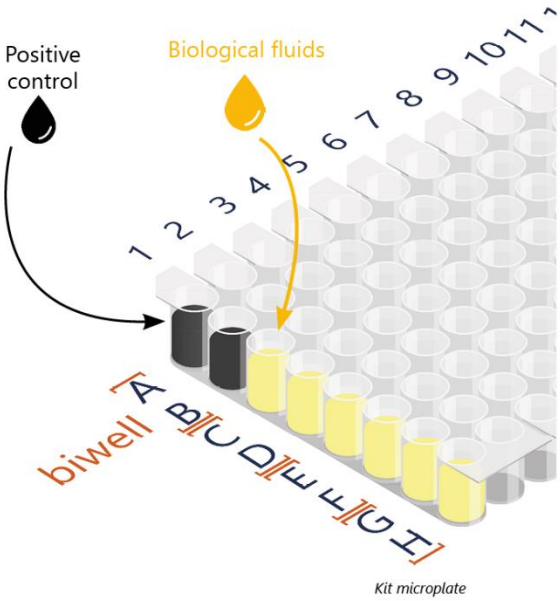
Notes*

Biological fluids protocol

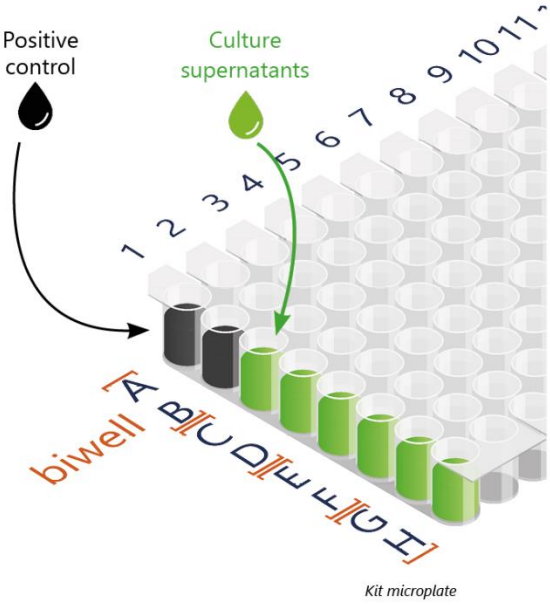
1 Distribute 100 µL of the diluted samples 1/2
Distribute 100 µL of the positive control



Kit microplate

Culture supernatants protocol

1 Distribute 100 µL of undiluted samples
Distribute 100 µL of the positive control



Kit microplate

Joint protocol

2 Add 100 µL of conjugate

3 Add 100 µL of TMB solution

4 Add 50 µL of stopping solution

5 Record optical densities

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

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