Instruction manual
BIOK291-CPAT_NO_(EN)_V03
03/06/2024

MONOSCREEN Ab ELISA

Monoscreen AbELISA *Clostridium perfringens* Alpha toxin

Référence : BIO K 291

ELISA kit for serodiagnosis of *Clostridium perfringens* Alpha toxin
Monowell, blocking

For veterinary *in vitro* use only

Sample / Dilution	All species
Serum – Plasma* / 2X	✓

*Hereafter, we will refer to it as serum.

Présentation

Product reference	BIO K 291/2
Format	2 plaques, barrettes de 8 puits
Réactions	192 tests

Kit composition

Provided material		BIO K 291/2
Microplate	Microplate	2
Washing solution	Washing solution (20X)	1 X 100 mL
Dilution solution	Colored dilution solution (1X)	1 X 60 mL
Conjugate	Conjugate (20X)	1 X 1,250 mL
CTL POS	Positive control	1 X 0,5 mL
CTL NEG	Negative control	1 x 0,5 mL
TMB solution	TMB solution (1X)	1 X 25 mL
Stop solution	Stopping solution (1X)	1 X 15 mL

Historique de révision

Date	Version	Modifications
03/06/2024	V03	Layout modification and simplification of the entire instruction manual.

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

A. Introduction

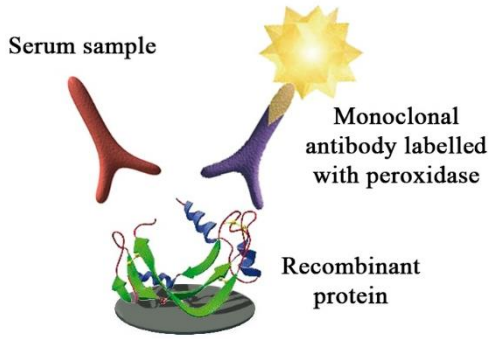
Enterotoxaemia is a fatal enteric disease that affects all species of domestic animals and is attributable to a toxigenic type of *Clostridium perfringens*. The latter is an anaerobic, strongly gram-positive bacterium that has the ability to form heat-resistant endospores. This bacterium is grouped into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

The BIO K 291 test is designed to monitor the animal's serological response after immunization by a vaccine or natural contact with *Clostridium perfringens*. As it is a blocking test, it can be used in all animal species.

B. Test principle

The 96-well microplate has been sensitized by a recombinant *Clostridium perfringens* alpha toxin. The operator deposits the previously diluted test sera and plasma in the microplate's wells. After 2 hours' incubation and a rinse step, the operator adds the conjugate, which is a specific monoclonal antibody against *Clostridium perfringens* alpha toxin coupled to peroxidase. After incubating and washing the preparation, the operator adds the chromogen tetramethylbenzidine (TMB). This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and not being carcinogenic. The intensity of the color is inversely proportionate to the sample's serum titre. Positive and negative controls are provided with the kit to be able to validate the test results.



C. Material required but 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
- Dilution microplate
- Incubator at 21±3°C
- Incubator at 37±2°C
- Standard laboratory equipment: graduated cylinder, tube rack, lid, …

D. Warnings and precautions of 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 the solutions

- The solutions are to be prepared extemporaneously.
- The washing solution must be diluted 20-fold in distilled/demineralized water. The solution crystallizes spontaneously when cold. 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 ready to use. The dilution is colored in yellow. It is used for dilution of samples, kit controls, and conjugate.
- The conjugate must be diluted 20-fold in the dilution solution.
- The stop solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Procedure

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

1. Distribute 50 µL/well of dilution solution 1X. add 50 µL/well of serum samples and of positive and negative control. Homogenize by pipetting up and down.

   Cover and incubate the plate at **37 ± 2°C** for **120 ± 5 min.**

N.B.: To avoid differences in incubation time between samples, sample dilutions and reference dilutions can be prepared in a dilution microplate (recommended dilution: 60 µL dilution solution + 60 µL sample) before transfer (100 µL) into the test microplate using a multi-channel pipette.

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 wells between each wash.
3. Distribute the **diluted conjugate** at a rate of **100 µL per well**. Cover with a lid and incubate the plate at **37 ± 2°C** for **30 ± 2 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 wells between each wash.
5. Distribute **100 µL** of **TMB solution** per well. Incubate at **21 ± 3°C** for **10 ± 1 min** in the dark, without covering.
6. Distribute the **stopping solution** at a rate of **50 µL** 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.

G. Validation of results

The test may be **validated** only of the following two conditions are met:

- OD neg – OD pos > 0,7
- % inh positive > 30%

H. Interpretation of results

- Measure the optical densities of the positive and negative control (OD pos and OD neg) and those of all the samples (OD samples).
- Calculate the percent inhibition (% inh) for each tested sample and the positive control by means of the following formulas:

$$\%inh\ sample = \frac{OD\ neg - OD\ sample}{OD\ neg}\ x\ 100$$

$$\%inh\ positive = \frac{OD\ neg - OD\ pos}{OD\ neg}\ x\ 100$$

Determine each sample's positivity using the scale shown in the table below.

	Results	Status
Sample	% inh < 20	0
	20 ≤ % inh < 40	+
	40 ≤ % inh < 60	++
	60 ≤ % inh < 80	+++
	80 ≤ % inh	++++

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ANALYSISCREEN

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SCAN ME

Notes*

1 Distribute 50 µL of dilution solution

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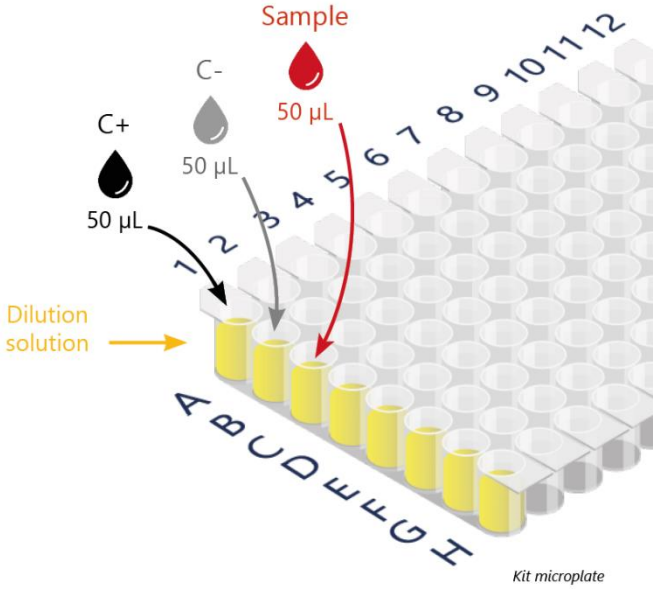
Add 50 µL of samples and 50 µL of positive and negative controls



Kit microplate

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 of use of which they are a summary.

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