MONOSCREEN Ab ELISA

Instructions for use
BIOK466-Besnoitia-besnoiti_NO_(EN)_V06
28/08/2024

Monoscreen AbELISA *Besnoitia besnoiti*

Reference : BIO K 466

ELISA Kit for serodiagnosis of Besnoitiosis
Monowell, blocking

In vitro and strictly veterinary use

Sample	Species	Individual analysis	Pool analysis*, possible up to
Sera and plasma	Cattle	✓	10

* This is done in accordance with the legislation in force in your country, the certifying body or the recommendations made by the NRL when they exist. Mixtures must be made volume to volume, i.e. by taking the same volume of each of the sera making up the mixture.

Presentation

Product reference	BIO K 466/2	BIO K 466/5
Format	2 plates, strip of 8 wells	5 plates, strips of 8 wells
Reactions	192 tests	480 tests

Composition of the kit

Provided material	BIO K 466/2	BIO K 466/5
Microplates	2	5
Washing solution (20X)	1 X 100 mL	1 x 250 mL
Colored dilution solution (1X)	1 X 60 mL	1 x 125 mL
Conjugate (50X)	1 X 0,6 mL	1 x 1,5 mL
Positive control	3 X 0,5 mL	4 x 1 mL
Negative control	3 X 0,5 mL	4 x 1 mL
Single component TMB (1X)	1 X 25 mL	1 x 55 mL
Stop solution (1X)	1 X 15 mL	1 x 30 mL

Revision history

Date	Version	Modifications
07/03/2022	V01	First version
15/03/2022	V02	Addition of columns “individual analysis” and “pool analysis*, possible up to”
19/09/2022	V03	Modification of the kit composition
01/09/2023	V04	Addition of pool analysis up to 10
21/02/2024	V05	Addition of 5 plates conditioning
28/08/2024	V06	Additional kit on demand

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

A. Introduction

Besnoitia besnoiti, the causative agent of bovine besnoitiosis, is an obligate intracellular protozoan. The disease affects mainly young cattle. Besnoitiosis is epizootic in the south of France, but is now widely distributed in Africa, Asia and in Southwestern Europe. The most likely pathway of transmission would be transcutaneous, by stinging insects (tabanids, stomox).

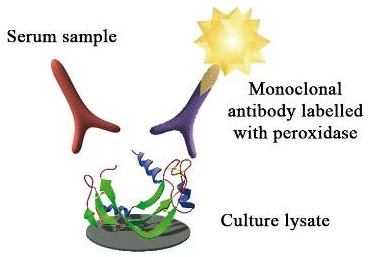
During infection, an incubation stage of 3 to 6 days is followed by 3 successive clinical stages:

- A febrile stage of 3 to 7 days; the tachyzoites multiplication in endothelial cells of blood vessels increases the animal temperature
- A second stage of 1 to 2 weeks; the bradyzoites cysts generate subcutaneous oedema
- A chronic stage of several months, characterized by alopecia and sclerodermia. The skin becomes then markedly thickened and wrinkled, and parasitic cysts are observed on conjunctiva and sclera. This ultimate phase leads generally to the death of the animal or to its euthanasia.

Serologic tests are available for the detection of the specific antibodies of *Besnoitia besnoiti* present in the chronic stage. In order to avoid the transfers contaminated animals and to control the spread of the bovine besnoitiosis, it is essential to use diagnostic tools that detect the pathogens at the early stages of the disease.

B. Test principle

The test uses 96-well microtitration plates sensitized by *besnoitia besnoiti*'s culture lysate. The operator deposits the previously diluted test sera in the microplate's wells. After 120 minutes' incubation and a washing step, the operator adds the conjugate, which is a specific monoclonal antibody against *Besnoitia besnoiti* 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 titer. Positive and negative control sera are provided with the kit to be able to validate the test results.



C. Additional material and required equipment (not provided)

- Distilled/demineralized water
- Graduated mono- or multichannel pipettes (2-20µL, 20-200µL et 100-1000µL range) and single-use tips
- Microplate reader (450nm filter)
- Microplate washer
- Incubator at 37±2°C
- Standard laboratory equipment: graduated cylinder, tube rack, lid, ...

Additional kit

- **Reference material: Besnoitia Besnoiti positive serum (Ref.: BDE K 466-1) available on demand.**

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 is ready to use. The dilution solution is colored in yellow. It is used for dilution of samples, positive and negative serums, and conjugate.
- The conjugate must be diluted 50-fold in the dilution solution.
- The stopping solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Preparation of the samples

- **Serum and plasma samples** and kit controls (positive and negative control) must be diluted **2-fold** in the dilution buffer and homogenized.
  Avoid using hemolyzed or coagulated samples.

  Recommended dilution:
  50µL of sample + 50µL of dilution solution.

G. Procedure

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

1. Distribute the **diluted samples** and **diluted kit controls** at a rate of **100µL** per well.
   Cover and incubate the plate at **37 ± 2°C** during **120 ± 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 wells and the desiccation of the microplate between each wash.
3. Add **100 µL** of **diluted conjugate** per well. Cover with a lid and incubate the plate at **37 ± 2°C** during **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 and the desiccation of the microplate between each wash.
5. Distribute **100 µL** of **TMB solution** per well.
   Incubate at **21 ± 3°C** during **10 ± 1 min** away from the light, without covering.
6. Distribute the **stopping solution** at rate of **50 µL** per well. Color 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 stopping solution.

H. Validation of results

The test can only be validated if :

- the difference between positive and negative serum optical density readings is greater than 0,700.

$$OD_{\text{negative serum}} - OD_{\text{positive serum}} > 0{,}700$$

- the positive serum's inhibition percentage (%inh) is greater than 50%.

$$\%inh_{\text{positive serum}} > 50\%$$

I. Interpretation of results

Calculate for each sample its inhibition percentage (%inh) using the following formula :

$$\%\ inh = \frac{OD_{\text{negative serum}} - OD_{\text{sample}}}{OD_{\text{negative serum}}} * 100$$

	Results	Status
Sample individual and pool of 10	%inh < 40%	Negative
	%inh ≥ 40 %	Positive

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

ANALYSISCREEN

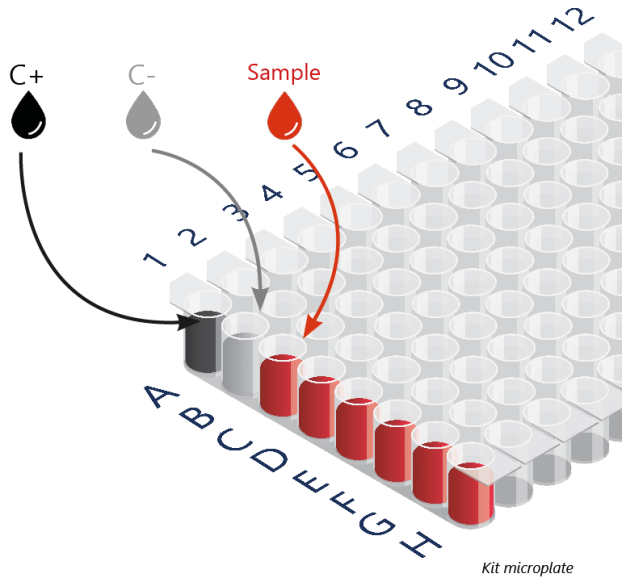
AnalysiScreen™ is the new module for reading and interpreting all types of Monoscreen™ and Multiscreen™ ELISA plates. AnalysiScreen™ is:

- 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 Distribute 100µL of the diluted samples (1/2) and diluted controls (1/2)



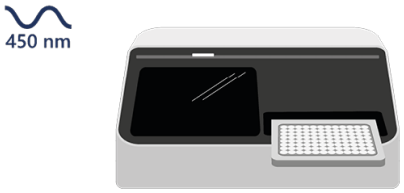
Kit microplate

2 Distribute 100 µL of diluted conjugate (1/50)

3 Distribute 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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