HANDBOOK

BioLisa® kit Besnoitia Ab

ELISA kit for the detection of antibodies to *Besnoitia besnoiti* in bovine individual serum

Method	Matrix	Species	Protocol
Indirect one-well ELISA	Individual serum	Bovine	Short incubation

Cat.No. : BIOLK007
BIOLK008
BIOLK009

MU/BES/00002/EN
Rev 10/20

biosellal

27 chemin des Peupliers
69570 Dardilly
FRANCE
+33 (0) 4 26 78 47 60
www.biosellal.com
contact@biosellal.com

For veterinary use only

General information

Besnoitia besnoiti is a protozoan parasite responsible of bovine besnoitiosis. This disease progresses in two sequential phases. First, a febrile acute phase with fever, oedemas and respiratory disorders, and a scleroderma phase characterized by the presence of subcutaneous tissue cysts and skin lesions leading to a high mortality level.
The parasite is transmitted the stomoxe bite.
Today, there is no effective treatment. The prevention and the regular screening of infected bovine are the best way against the disease spreading.

BioLisa® kit Besnoitia Ab is a sensitive and specific indirect one-well ELISA for the detection of antibodies to *Besnoitia besnoiti*, in individual bovine serum samples.

Principle of the test

The plates are coated with a *Besnoitia besnoiti* tachyzoïtis preparation. During the sample incubation, the anti-*Besnoitia* antibodies bind to the antigen. After a washing step which removes all the no binded proteins, an HRP conjugated anti-bovine is added. This conjugate specifically binds the bovine antibodies which binded to the well (positive sample). The unbound conjugate is removed by washing, and the substrate solution is added. By contact with the HRP enzyme, the substrate solution becomes blue. After stopping the reaction, the colour turns to yellow. The optical density (OD) is measured by a spectrophotometer. The coloration in the well indicate the presence of a positive sample.

Kit cotent

Component		Quantity		
		Cat.No BIOLK007	Cat.No BIOLK008	Cat.No BIOLK009
1	Test plate 12 strips of 8 wells per plate	1	2	5
2	Sample Diluent	1 x 30mL	1 x 60mL	2 x 80mL
3	Negative Control	1 x 0.12mL	1 x 0.25mL	1 x 0.6mL
4	Positive Control	1 x 0.12mL	1 x 0.25mL	1 x 0.6mL
5	Wash buffer 10X	1 x 125mL	1 x 125mL	2 x 125mL
6	Conjugate 100X	1 x 0.15mL	1 x 0.3mL	1 x 0.6mL
7	Conjugate Diluent	1 x 15mL	1 x 30mL	1 x 60mL
8	Substrate	1 x 15mL	1 x 30mL	1 x 60mL
9	Stop Solution	1 x 15mL	1 x 30mL	1 x 60mL
Certificate of analysis				
Adhesive plate covers				

The components must be stored at 5±3°C and are stable until the expiration date indicated on the labels. The unused plate strips must be stored in the sealed bag with a desicate bag at 5±3°C. The test strips can be stored for at least 6 weeks after opening the plate bag.

Symbols


Legal manufacturer

Batch number


Use by date


Temperature limitations for storage

Catalog number


Protect from light


For cattle samples

Not provided material

- Monochannel and multichannels pipettes
- Single-use tips
- Microplate absorbance reader with a 450nm filter
- Distilled or deionised water
- Single-use containers
- Pre-dilution plates

General precautions

Always wear appropriate personal protective equipment. For more information, please consult the appropriate material safety data sheets (MSDS) (available on request).

- Carefully read and strictly follow the instruction of use
- Handle all materials according to the Good Laboratory Practices
- Consider all the reagents and samples as potentially contaminated
- All unused biological materials should be disposed according to the local, regional and national regulations.
- Do not use reagents beyond expiration date.
- Do not mix components from different kit batches.
- Incude positive and negative controls on each plate or test strip series.

Before starting

All reagents should equilibrate to room temperature (21±4°C) before use.

Wash solution : the wash solution 10X must be 10 fold diluted in distilled/deionised water.
Example : for one plate, dilute 50mL of wash solution 10X into 450mL of water.
The diluted wash solution could be stored for one month at 5±3°C.

Conjugate : the conjugate 100X must be 100 fold diluted in the conjugate diluent. *Example : for one plate, dilute 100µL of conjugate 100X into 10mL of diluent.*
The diluted conjugate solution must be used during the day after dilution.

Samples: fresh, refrigerated or frozen individual sera could be used.

Protocol

1. Sample dilution 1:20 :

In the test plate, add 95µL of sample diluent into each well.
Add 5µL of positive control (PC) and negative control (NC) into the appropriate wells *(A1B1 and C1D1 or example).*
Add 5µL of samples into the following wells.

Gently shake and cover the plate. Incubate **2h±12min at 37±3°C.**

2. Washing :

Empty the wells and **wash 3 times with 300µL** of diluted wash solution. After the last washing step, empty the plate and softly tap it on an absorbant paper to remove all the liquid.

This step could be done manually or with a plate washer

3. Conjugate

Add 100µL of diluted conjugate in each well.

Cover the plate and incubate **1h±6min at 37±3°C.**

4. Washing :

Repeat the step **3. Washing**

5. Revelation :

Add 100µL of substrate in each well.

Incubate the plate **10±1min at room temperature (21±4°C) in darkness.**

6. Stopping the reaction:

Stop the reaction by adding 100µL of stop solution in each well following the same order than with the substrate.

7. Reading :

Measure the optical density (OD) in a microplate reader at **450nm** within 20min after stopping the reaction. Make sure there is no dust or bubbles into the wells.

• Validation criteria

The results are validated if :

- The ratio (Mean PC OD / Mean NC OD) must be > 2
- The mean OD of the positive control (PC OD) is > 0.6

If one of these criteria is not compliant, the assay should be repeated.

• Calculations

Calculate the mean OD for the positive and negative controls.

The **percentage of positivity S/P** value is calculated for each sample, using the following formula :

$$S/P = \frac{\text{Sample OD - Mean NC OD}}{\text{Mean PC OD - Mean NC OD}} \times 100$$

• Interprtation of results

Result	Interpretation
S/P < 30 %	Negative
S/P ≥ 30 %	Positive