HANDBOOK

BioLisa® kit Neospora Ab

ELISA kit for the detection of antibodies to *Neospora caninum* in ruminant indiviual serum

Method	Matrix	Species	Protocol
Indirect ELISA	Individual serum	Cattle Sheep Goat	Short incubation

Information : modifications from previous version are indicated by a yellow highlighting

Cat.No. : BIOLK005
BIOLK006

MU/NEO/003/EN
Rev 06/20

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For veterinary use only

General information

Neospora caninum is one of the main cause of bovine abortion and neonatal morbidity, and also in some other ruminant species such as ovine and caprine. This worldwild spread parasite could be horizontaly transmited, but also during gestation due to the capacity of the parasite to cross the transplacental barrier. Neither treatment nor effective vaccination are currently available for this disease. This parasite could be found in temporary host, within 2 main forms : tachyzoïtis and bradyzoïtis. Lots of animals, including mamal pets such as dogs, could be hosts and contribute to the parasite spread.

BioLisa® kit Neospora Ab is a sensitive and specific indirect ELISA test for the detection of antibodies to *Neospora caninum*, in individual serum samples of bovine, ovine and caprine.

Principle of the test

The plates are coated with a *Neospora caninum* tachyzoïtis preparation. During the sample incubation, the anti-*Neospora* antibodies bind to the antigen. After a washing step which removes all the no binded proteins, an HRP conjugated anti-ruminant is added. This conjugate specifically binds to the ruminant 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 content

Component		Quantity	
		Cat.No BIOLK005	Cat.No BIOLK006
1	Test plate. 12 strips of 8 wells per plate	1	2
2	Sample diluent	1 x 30mL	1 x 60mL
3	Negative Control	1 x 0.12mL	1 x 0.24mL
4	Positive Control	1 x 0.12mL	1 x 0.24mL
5	Wash buffer 10X	1 x 125mL	1 x 125mL
6	Conjugate 100X	1 x 0.15mL	1 x 0.3mL
7	Conjugate diluent	1 x 15mL	1 x 30mL
8	TMB Substrate	1 x 15mL	1 x 30mL
9	Stop Solution	1 x 15mL	1 x 30mL
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, sheep and goat 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) (availables 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.
- Include 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 pre-dilution 1:20 :

In a pre-dilution plate, add 95µL of sample diluent into each well.
Add 5µL of positive control (PC) and negative control (NC) into the appropriated wells *(A1B1 and C1D1 for example).*
Add 5µL of samples into the following wells.

Gently shake the plate.

2. Sample distribution (finale dilution 1:200) :

In the test plate, add 10µL of 1:20 pre-diluted samples and controls into the wells.
Add 90µL of sample diluent into each well.

Gently shake and cover the plate. Incubate **1 hour ±6min at room temperature (21±4°C).**

3. 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.

4. Conjugate

Add 100µL of diluted conjugate in each well.

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

5. Washing :

Repeat the step **3. Washing**.

6. Revelation :

Add 100µL of substrate in each well.

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

7. Stopping the reaction :

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

8. Reading :

Measure the optical density (OD) in a microplate reader at **450nm** within 30min 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 > 3
- 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 :

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

• INTERPRETATION OF RESULTS

RESULT	INTERPRETATION
% S/P < 25%	Negative
25% ≤ % S/P < 50%	Positive +
50 % ≤ % S/P	Positive ++