EN

MONOSCREEN Ab ELISA

User manual

BIO K 451_NO_(EN)_V03
04/09/2024

MonoScreen AbELISA *Neospora caninum* EASY

Reference : BIO K 451

ELISA Kit for the serodiagnosis of bovine neosporosis

Monowell, indirect test

For *in vitro* and strictly veterinary use

Sample	Species
Blood serum	Bovine
Individual milk (skimmed* and non-skimmed)	Bovine

* 20 min. 4000 g centrifugation

To order

Product reference	BIO K 451/5
Format	5 plates, 8-wells strip
Reactions	480 tests

Kit composition

	BIO K 451/5
Microplate	5
Washing solution (20X)	1 X 250 ml
Colored dilution buffer (1X)	2 X 100 ml
Conjugate (50X)	1 X 1,5 ml
Reference - Positive serum - serum (black cap)	1 X 0,5 ml
Reference - Positive serum - milk (yellow cap)	1 X 0,5 ml
Reference - Negative serum	1 X 0,5 ml
Reference - Tracer	1 X 0,5 ml
Single component TMB solution (1X)	1 X 55 ml
Stopping solution (1X)	1 X 30 ml

Revision history

n/a	first version
V1.1.0	addition of the «individual milk» matrix
V1.2.0	amendment to item «G. Validation of results»
V02	removal of item «BIO K 451/2»
V03	modification of conjugate volume from 1,4 to 1,5 mL

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

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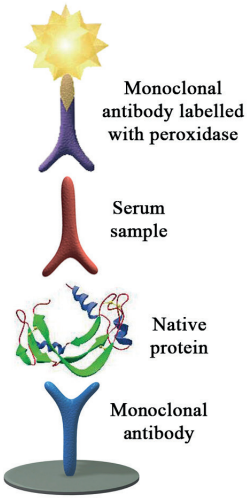
A. Introduction

Neospora caninum is a protozoan initially described as a parasite of the dog in which it is responsible for myositis and encephalitis. Bovine neosporosis is now recognized as a major cause of abortion in cattle. It is strongly suspected in 20% of farms with repeated abortion and a seropositive cow for *Neospora caninum* is 3 times more likely to have an abortion than a seronegative cow. Vertical transmission is standard (at least 80% of calves from seropositive cows are contaminated).

B. Test principle

96-well microplates were sensitized by a specific monoclonal antibody of a Neospora caninum protein. The antibody ensures the capture and purification of this protein from a protozoan lysate.

Blood sera and milks are diluted in the dilution buffer. After incubation and washing of the preparation, the conjugate is added, a specific monoclonal antibody anti-bovine IgG1 coupled with peroxidase. At the end of a second incubation of 30 minutes at 21±3°C and a second wash, the revelation solution is added (single component TMB solution). If specific immunoglobulins anti-*Neospora caninum* are present in the serum or milk, the conjugate remains attached to the well containing the protozoan and the enzyme catalyzes the transformation of colorless chromogene into a blue product. The intensity of the colouring is proportional to the specific antibody content in the sample.



C. Additional material and required equipment (not provided)

- Distilled/demineralized water
- Graduated mono- or multichannel pipettes (2-20µl, 20-200µl and 100-1000µl range) and single-use tips
- Microplate reader (450nm filter)
- Microplate washer and shaker (optional)
- Dilution microplate
- Standard laboratory equipment : graduated cylinder, tube rack, lid, ...

D. Precautions for use

- The reagents must be kept between +2 and +8°C. The wash solution may be stored at room temperature.
- Unused strips must be stored with the desiccant in the hermetically sealed aluminium 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 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 buffer is ready to use. The dilution buffer is coloured in yellow.
- The conjugate must be diluted 50-fold in the dilution buffer.
- The stopping solution is ready to use.
- La TMB solution is ready to use. It must be perfectly colourless.

F. Procedure

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

N.B. *: To avoid differences in incubation time between samples, sample dilutions and reference dilutions can be prepared in a dilution microplate before transfer (200 µl) into the test microplate using a multi-channel pipette.*

Serum protocol (1/20 dilution)

1. Distribute 190 µl/well of dilution buffer. Add 10 µl of serum and references per well. Homogenize by pipetting up and down.
2. Cover and incubate the plate at **21 ± 3°C** during **30 ± 3 min**.
3. 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 dessication of the microplate between each wash.
4. Add **100 µl of diluted conjugate** per well. Cover with a lid and incubate the plate at **21 ± 3°C** during **30 ± 3 min**.

Milk protocol (1/4 dilution)

1. For milk (1/4 dilution): distribute the dilution solution at rate of 150 µl per well. Add samples at rate of 50 µl per well. Homogenize by pipetting up and down.

   For the references (1/20 dilution): distribute 190 µl of dilution buffer per well. Add 10µl per well of reference. Homogenize by pipetting up and down.
2. Cover and incubate the plate at **21 ± 3°C** during **60 ± 5 min**.
3. 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 dessication of the microplate between each wash.
4. Add **100 µl of diluted conjugate** per well. Cover with a lid and incubate the plate at **21 ± 3°C** during **60 ± 5 min**.

Joint protocol

5. 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 dessication of the microplate between each wash.
6. Distribute **100 µl of TMB solution** per well.
7. Incubate at **21 ± 3°C** during **10 ± 1 min** away from the light, without covering.
8. Distribute the stopping solution at rate of **50 µl per well**. Colour changes from blue to yellow.
9. Record the optical densities using a plate spectrophotometer with a 450 nm filter within 5 minutes after adding the stopping solution.

G. Validation of results

The test can only be validated if :

- the difference between positive and negative serum optical density readings is greather than 0,450.

$$OD_{\text{positive serum (serum or milk)}} - OD_{\text{negative serum}} > 0{,}450$$

- the negative serum gives an optical density of less than 0,400.

$$OD_{\text{negative serum}} < 0{,}400$$

H. Interpretation of results

Calculate for each sample its coefficient (S/P %) using the following formula:

$$S/P\ \% = \frac{OD_{\text{sample}} - OD_{\text{negative serum}}}{OD_{\text{positive serum (serum or milk)}} - OD_{\text{negative serum}}} * 100$$

	Results	Status
Serum	S/P % < 70 %	Negative
	S/P % ≥ 70 %	Positive
Milk	S/P % < 50 %	Negative
	S/P % ≥ 50 %	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

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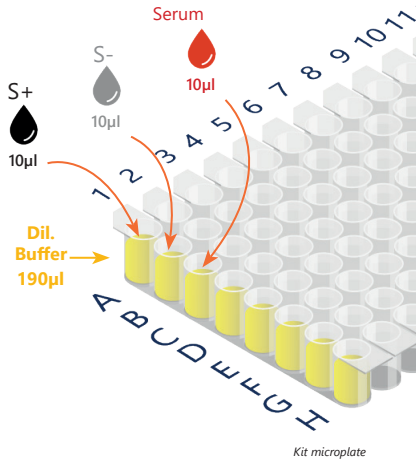
Notes*

Serum protocol

190 µl of dilution buffer + 10 µl of serum (1/20)
190 µl of dilution buffer + 10 µl of references (1/20)

Room Temp. — 30 min — 3X Wash



Kit microplate

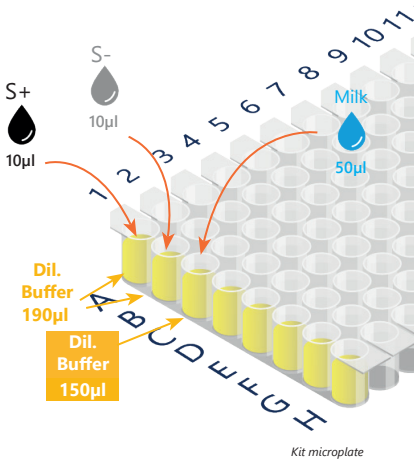
Add 100 µl of diluted conjugate (1/50 dilution)

Room Temp. — 30 min — 3X Wash

Milk protocol

150 µl of dilution buffer + 50 µl of milk (1/4)
190 µl of dilution buffer + 10 µl of references (1/20)

Room Temp. — 60 min — 3X Wash



Kit microplate

Add 100 µl of diluted conjugate (1/50 dilution)

Room Temp. — 60 min — 3X Wash

Add 100 µl of TMB solution

Room Temp. — 10 min — Obscurity

Add 50 µl of stopping solution

Record the optical densities

450 nm

* The notes do not replace the instructions of use of which they are a summary.