MONOSCREEN Ab ELISA

Instruction manual
BIOK451-Neo Easy_NO_(EN)_V04
26/11/2024

Monoscreen AbELISA *Neospora caninum Easy*

Reference : BIO K 451

ELISA test for serodiagnosis of bovine Neosporosis

Monowell, indirect test

For veterinary *in vitro* use only

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

*20 min. 4000g centrifugation.

Presentation

Product reference	BIO K 451/5
Format	5 plates, strips of 8 wells
Reactions	480 tests

Kit composition

Matériel fourni		BIO K 451/5
Microplate	Microplates	5
Washing solution	Washing solution (20X)	1 x 250 mL
Dilution solution	Colored dilution solution (1X)	2 x 100 mL
TMB solution	Single component TMB (1X)	1 x 55 mL
Stop solution	Stopping solution (1X)	1 x 55 mL
Conjugate	Conjugate (50X)	1 x 1,5 mL
CTL POS serum	Positive control serum (black cap)	1 x 0,5 mL
CTL POS milk	Positive control milk (yellow cap)	1 x 0,5 mL
CTL NEG	Negative control	1 x 0,5 mL
Tracer	Tracer	1 x 0,5 mL

Revision history

Date	Version	Modifications
31/08/2022	V02	Suppression of BIO K 451/2
20/09/2023	V03	Modifications of reference names.
26/11/2024	V04	Modification of the volume of conjugates and stop solution.

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

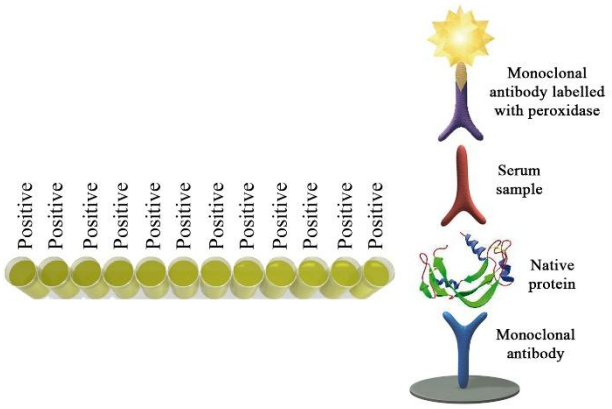
A. Introduction

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 solution. 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 chromogen into a blue product. The intensity of the coloring is proportional to the specific antibody content in the sample.



C. Material required but not provided

- Distilled/demineralized water.
- Dilution microplates.
- Graduated mono or multichannel pipettes (2-20 µL, 20-200 µL and 10-1000 µL range) and single-use tips.
- Microplate washer (optional).
- Microplate reader (450nm filter).
- Incubator at 21±3°C.
- Standard laboratory equipment: graduated cylinder, tube rack, lid,...
- Dilution microplate.

D. Warnings and precautions of use

- The reagents must be kept between +2 and +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 1M 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 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 controls, tracer and conjugates.
- 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. 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, samples dilution and controls dilution 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 solution. Add 10 µL of serum and controls per well. Homogenize by pipetting up and down.
2. Cover and incubate the plate at **21±3°C** during **30±3min**.
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 desiccation of the microplate between each wash.
4. Add **100 µL of diluted conjugate** per well. Cover and incubate the plate at **21±3°C** during **30±3min**.

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 a rate of 50 µL per well. Homogenize by pipetting up and down.
   For the controls (1/20 dilution): distribute 190 µL of dilution solution per well. Add 10 µL per well of controls. Homogenize by pipetting up and down.
2. Cover and incubate the plate at **21±3°C** during **60±5min**.
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 desiccation of the microplate between each wash.
4. Add **100 µL of diluted conjugate** per well. Cover 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 desiccation of the microplate between each wash.
6. Distribute **100 µL of TMB solution** per well.
   Incubate at **21±3°C** during **10±1 min** away from the light, without covering.
7. Distribute the **stopping solution** at a rate of **100 µL per well**. Color changes from blue to yellow.

Tip: Lightly tap the frame of the plate to homogenize it after adding the stopping solution.

8. 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 control optical density readings is greater than 0,450.

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

- The negative control gives an optical density of less than 0,400.

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

H. Interpretation of results

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

$$S/P\,(\%) = \frac{OD\ sample - OD\ negative\ control}{OD\ postitive\ control_{\ (serum\ or\ milk)} - OD\ negative\ control} * 100$$

		Results	Status
IgG1	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

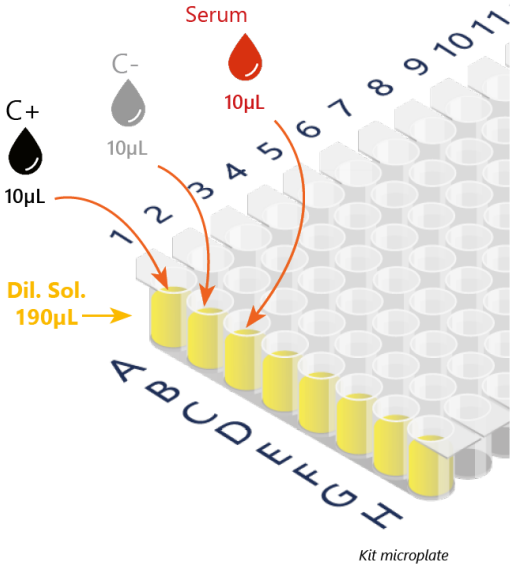
SCAN ME

Notes*

Serum protocol

1 Sample dilution 1/20
C+ C- and serum dilution 1/20

Room Temp. — 30 min → 3X Wash



Kit microplate

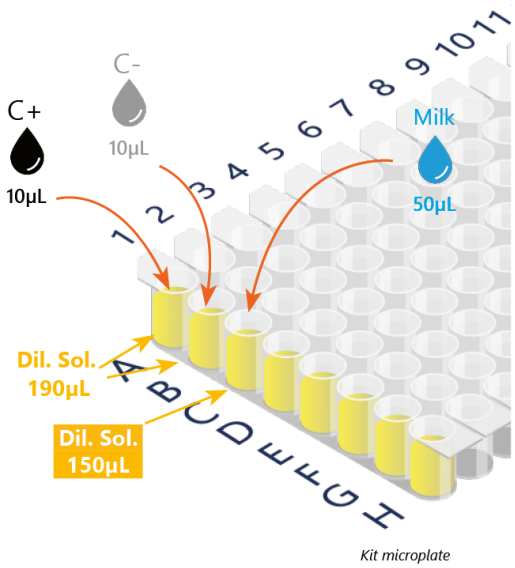
2 Add 100 µL of conjugate

Room Temp. — 30 min → 3X Wash

Milk protocol

1 Sample dilution 1/4
C+ C- and milk dilution 1/20

Room Temp. — 60 min → 3X Wash



Kit microplate

2 Add 100 µL of conjugate

Room Temp. — 60 min → 3X Wash

Joint protocol

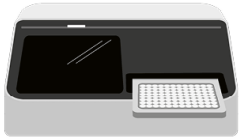
3 Add 100 µL of TMB solution

Room Temp. — 10 min — Obscurity

4 Add 100 µL of stopping solution

5 Record optical densities

450 nm



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

Contact us
support.immuno@biox.com
www.biox.com +32 (0) 84 32 23 77
Bio-X Diagnostics
38, rue de la Calestienne
5580 Rochefort (Belgium)