MONOSCREEN Ab ELISA

Instruction manual
BIOK451-Neo-Easy_NO_(EN)_V07
07/01/2026

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	Ruminant***
Individual milk (skimmed* and non-skimmed)	Bovine
Blotting paper**	Bovine

*20 min. 4000g centrifugation.
**Requires a larger amount of dilution solution; contact Bio-X if necessary.
***The Perox Moab a-IgG1 bovine conjugate used in the kit also recognizes antibodies from goats and sheep.
The kit can therefore be used on sera from small ruminants (contact us).

Presentation

Product reference	BIO K 451/2	BIO K 451/5
Format	2 plates, strips of 8 wells	5 plates, strips of 8 wells
Reactions	192 tests	480 tests

Kit composition

Provided material		Type*	Code	BIO K 451/2	Code	BIO K 451/5
Microplate	Microplates	1	D01276	2	D01276	5
Washing solution	Washing solution (20X)	A	D00695	1 X 100 mL	D00696	1 X 250 mL
Dilution solution	Colored dilution solution (1X)	A	D01511	1 X 125 mL	D01555	1 X 250 mL
Conjugate anti-IgG1	Conjugate anti-IgG1 (50X) (blue cap)	1	D01476	1 X 0,6 mL	D01477	1 X 1,5 mL
Conjugate anti-IgG2	Conjugate anti-IgG2 (50X) (green cap)	1	D01597	1 X 0,6 mL	D01554	1 X 1,5 mL
CTL POS serum IgG1	Positive control serum IgG1 (black cap)	a	D01416	1 X 0,5 mL	D01416	1 X 0,5 mL
CTL POS serum IgG2	Positive control serum IgG2 (purple cap)	a	D01552	1 X 0,5 mL	D01552	1 X 0,5 mL
CTL POS milk	Positive control milk (yellow cap)	a	D01415	1 X 0,5 mL	D01415	1 X 0,5 mL
CTL NEG	Negative control (white cap)	a	D01123	1 X 0,5 mL	D01123	1 X 0,5 mL
TMB solution	Single component TMB (1X)	A	D01585	1 X 30 mL	D01557	1 X 60 mL
Stop solution	Stopping solution (1X)	A	D00680	1 X 30 mL	D01556	1 X 60 mL

* : (1) : dependent on kit and batch / (a) : dependent on kit / (A) : substitutable with components A (B): substitutable with components B.

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.
15/01/2025	V05	Conjugate IgG2 added.
01/10/2025	V06	Addition of blotting paper matrix and adjustment of component volume.
07/01/2026	V07	Addition of 2-plate packaging.

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

A. Introduction

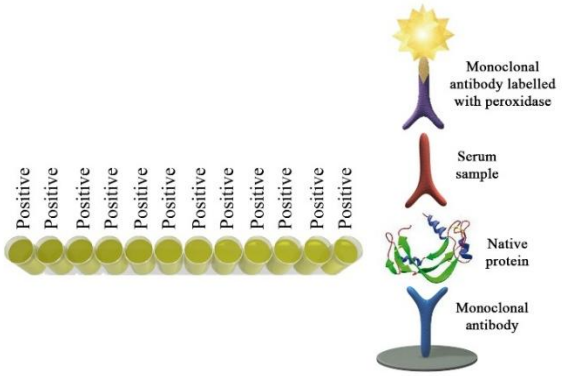
Neospora caninum is a protozoan initially described as a parasite of the dog in which it is responsible for myositis and encephalitis. Bovine neosporosis is now recognized as a major cause of abortion in cattle. It is strongly suspected in 20% of farms with repeated abortion and a seropositive cow for *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).

Generally speaking, in cattle, syndesmochorion placentation prevents the transfer of immunoglobulins from the cow to her offspring during gestation. Furthermore, at udder level, only Ig G1 is transferred to the colostrum*. Some pathogens, such as *Neospora caninum*, are able to cross the placental barrier and come into contact with the bovine fetus. If the calf is immunocompetent at the time of infestation, it will produce specific Ig G2 antibodies against the pathogen encountered. These can then be measured using specific serological tests (ELISA). A positive result indicates active circulation of the pathogen and vertical transmission in the herd.

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 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.



IgG2

For Neosporosis control, IgG2 ELISA appears to be a reliable and practical means of distinguishing vertically infected calves (0-1 months)**.

From: Neosporosis: how IgG2 ELISA could contribute in the diagnosis of vertically infected calves?

EVRARD J., DELOOZ L., GREGOIRE F.

ARSIA (Regional Association for Animal Identification and Health) Ciney, Belgium

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 (450 nm filter).
- Incubator at 21±3°C.
- Standard laboratory equipment: graduated cylinder, tube rack, lid,...
- Dilution microplate.

Additional kits

- **Tracer Neospora IgG1 (Ref. : BDE K 451-1).** Internal reference material for neosporosis serology by ELISA.
- **Tracer Neospora IgG2 (Ref. : BDE K 451-2).** Internal reference material for neosporosis serology by ELISA.

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.
- 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 conjugates (IgG1 & IgG2) 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** (IgG1 or IgG2) 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.
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
	Blotting paper	S/P % < 40 %	Negative
		40 % ≤ S/P % < 70 %	Doubtful
		S/P % ≥ 70 %	Positive
IgG2	**Serum**	S/P % < 30 %	Negative
		S/P % ≥ 30 %	Positive
	Blotting paper	S/P % < 20 %	Negative
		20 % ≤ S/P % < 30 %	Doubtful
		S/P % ≥ 30 %	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

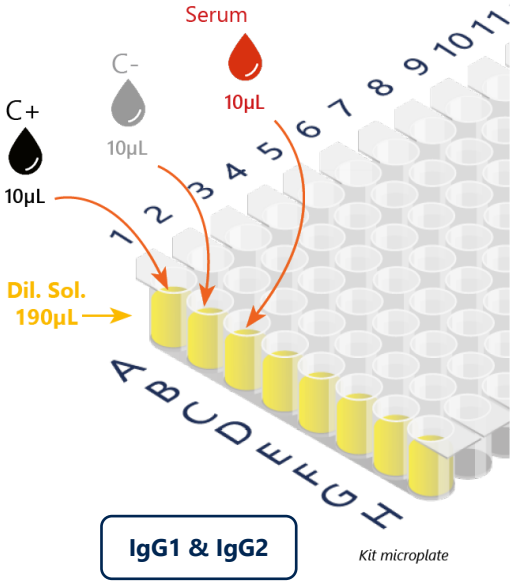
SCAN ME

Notes*

Serum protocol

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

Room Temp. — 30 min → 3X Wash



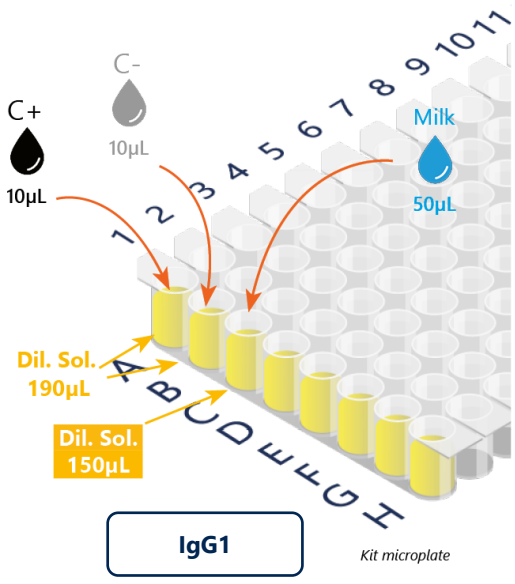
2 Add 100 µL of conjugate

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



2 Add 100 µL of conjugate

Room Temp. — 60 min → 3X Wash

Joint protocol

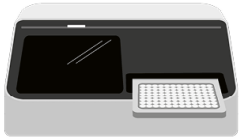
3 Add 100 µL of TMB solution

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.

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