General Information

This diagnostic kit is designed to detect antibodies directed against *Neospora caninum* in bovine milk (individual and bulk milks) by indirect ELISA.

Description and Principle

Wells are coated with purified *Neospora caninum* extract.

Samples to be tested and controls are added to the microwells. Anti-*Neospora* antibodies, if present, form an antibody-antigen complex.

An anti-ruminant-peroxidase (HRP) conjugate is added to the microwells. It fixes to the anti-*Neospora* antibodies, forming an antigen-antibody-conjugate-HRP complex.

After washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested:

- In the presence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution.
- In the absence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Kit Components

Reagents*
Microplates coated with purified *Neospora caninum* extract
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 3
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0.5 M)

** Quantities supplied are indicated on the kit label.*

1. The conjugate, the controls and the substrate solution must be stored at 5°C (± 3°C).
2. The other reagents can be stored between +2°C and +26°C.
3. Components bearing the same name (*wash solution, dilution buffers*) can be used for the entire IDvet product range.

Materials required but not provided

1. Mono or multi-channel micropipettors capable of delivering volumes of 10 µl, 100 µl, and 200 µl.
2. Disposable tips.
3. 96-well microplate reader.
4. Distilled or deionized water.
5. Manual or automatic wash system.

Precautions

1. Do not pipette by mouth.
2. The substrate solution can be irritating to the skin.
3. The stop solution (0,5 M) may be harmful if swallowed. It may cause sensitisation by skin contact (**R22-43**). Avoid contact with skin (**S24-37**).
4. Do not expose the substrate solution to bright light nor to oxidating agents.
5. Decontaminate all reagents before elimination.

Samples Preparation

In order to avoid differences in incubation times between specimens, it is possible to prepare a 96-well plate containing the test and control specimens, before transferring them into an ELISA microplate using a multichannel pipette.

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature and mix thoroughly to ensure that the Wash Concentrate is completely solubilized.

Prepare the Wash Solution **(1X)** by diluting the Wash Concentrate **(20X)** in distilled/deionized water.

Testing Procedure

Allow all the reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or Vortex.

Milk samples:

Centrifuge each whole milk sample, or just let the samples sit, so that the cream separates from the lactoserum (cream on the top, lactoserum on the bottom):

Pipette under the cream so that only the lactoserum enters the cone (antibodies are found in the lactoserum).

1. Add :
   - 100 µl of **Negative Control** to wells A1 and B1.
   - 100 µl of **Positive Control** to wells C1 and D1.
   - 100 µl of each milk sample to be tested to the remaining wells.
2. Incubate **45 min** ± **4 min** at 21°C (± 5°C) (short incubation) or **overnight** (for 16 to 20 hours) at 4°C (± 2°C).
3. Empty the wells. Wash each well 3 times with approximately 300 µl of the **Wash Solution.** Avoid drying of the wells between washings. *Be careful that there is no fatty ring left in the well after washing. To avoid fat residues, it is possible to include a soaking time of 2 – 5 minutes between washes.*
4. Prepare the **Conjugate 1X** by diluting the **Concentrated Conjugate 10X** to 1/10 (short incubation) or to 1/20 (overnight incubation) in **Dilution Buffer 3.**
5. Add 100 µl of the **Conjugate 1X** to each well.
6. Incubate **30 min** ± **3 min** at 21°C (± 5°C).
7. Empty the wells. Wash each well 3 times with approximately 300 µl of the **Wash Solution.** Avoid drying of the wells between washings.
8. Add 100 µl of the **Substrate Solution** to each well.
9. Incubate **15 min** ± **2 min** at 21°C (± 5°C) in the dark.
10. Add 100 µl of the **Stop Solution** to each well in order to stop the reaction.
11. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean value of the Positive Control O.D. (OD_{PC}) is greater than 0.350.

$OD_{PC} > 0.350$

✓ the ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is greater than 3.

$OD_{PC} / OD_{NC} > 3$

Interpretation

For each sample, calculate the S/P percentage (S/P%): sample value (OD_{sample}) divided by the mean Positive control value (OD_{PC}) multiplied by 100:

$$S/P\ \% = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100$$

Samples with a S/P%:

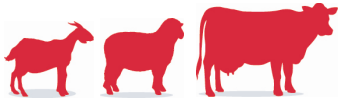
INDIVIDUAL MILK	
S/P % ≤ 30%	NEGATIVE
S/P % > 30%	POSITIVE
BULK MILK	
S/P % ≤ 20%	NEGATIVE
S/P % > 20%	POSITIVE

Note: the overnight incubation allows for improved test sensitivity.

IDvet
Innovative Diagnostics

Certified management system

ID Screen®
Neospora caninum Milk Indirect



Kit for the detection of specific *N. caninum* antibodies by indirect ELISA in bovine milk (individual and bulk milks).

Short and overnight incubation

For *in vitro* use

NCSMILK 1114 GB

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