

NEOSPORA CANINUM ELISA KIT

ELISA kit for serodiagnosis of *Neospora caninum*

Indirect test for blood sera and milk

Diagnostic test for cattle

I - INTRODUCTION

Neospora caninum is a protozoan that was originally described as a parasite in dogs, in which it causes myositis and encephalitis. Bovine neosporosis is now recognised as a major cause of spontaneous abortion in cattle. It is highly suspected on 20% of the farms with repeated abortions and a cow that is seropositive for *Neospora caninum* has a threefold greater risk of aborting than a cow that is *Neospora*-negative. *Neospora* is responsible for 21% of spontaneous abortions occurring in an individual animal. This percentage rises to 33% for the herd as a whole. Vertical transmission is the rule (at least 80% of the calves born to seropositive cows are infected). Serotesting before the calf's first colostrum intake will reveal prenatal infection.

II - PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by a purified *Neospora caninum* protein. The plate's odd columns (1, 3, 5, 7, 9 and 11) contain the purified protein, whereas the even columns (2, 4, 6, 8, 10 and 12) contain a control antigen. We thus have a genuine negative control. Using such a control reduces the number of false positives considerably.

The test blood sera or milks are diluted in the buffer for dilution. The plate is incubated and washed, then the conjugate, a peroxidase-labelled anti-bovine IgG1 monoclonal antibody, is added to the wells. The plate is then incubated a second time at 21°C +/- 3°C washed again and the chromogen tetramethylbenzidine (TMB) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific anti-*Neospora caninum* immunoglobulins are present in the test sera or milks the conjugate remains bound to the microwell that contains the protozoan and the enzyme catalyses the transformation of the colorless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of specific antibody in the sample. The signal read off the negative control microwell is subtracted from that of the positive microwell sensitised by the protozoan protein.

III - COMPOSITION OF THE KIT

- **Microplates:** 96-well microtitration plates (6 strips of 16 wells). The odd columns (1, 3, 5, 7, 9 and 11) are sensitised by purified protein from *Neospora caninum* and the even columns (2, 4, 6, 8, 10 and 12) by the control antigen.
- **Washing solution:** One bottle of 20x concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to 21°C +/- 3°C until all crystals have disappeared. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water.

- **Dilution buffer:** One bottle of 5x concentrated buffer for diluting the blood sera, milks and conjugate. The bottle's content is to be diluted with distilled or demineralised water. If a deposit forms at the bottom of the receptacle filter the solution on Whatman filter paper.
- **Conjugate:** 1 bottle of anti-bovine immunoglobulin-peroxidase conjugate (horseradish peroxidase-labelled anti-bovine IgG1 monoclonal antibody).
- **Positive serum:** One bottle of positive serum. Reconstitute this serum with 0.5 ml of distilled or demineralised water. The reconstituted serum may be kept at -20°C. Divide the reconstituted serum into several portions before freezing in order to avoid repeated freezing and thawing. If these precautions are taken the reagent may be kept for several months.
- **Single component TMB:** One bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. **This solution is ready to use.**
- **Stop solution:** One bottle of the 1 M phosphoric acid stop solution.

	BIO K 192/2	BIO K 192/5
Microplates	2	5
Washing solution	1 X 100 ml (20 X)	1 x 250 ml (20x)
Dilution buffer	1 X 50 ml (5 X)	1 x 100 ml (5 X)
Conjugate	1 X 0.5 ml (50 X)	1 X 1.4 ml (50 X)
Positive serum	1 X 0,5 ml (1 X) freeze-dried	1 X 0,5 ml (1 X) freeze-dried
Single component TMB	1 X 25 ml (1 X)	1 x 55 ml (1 X)
Stop solution	1 X 15 ml (1 X)	1 x 30 ml (1 X)

IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

Distilled water, graduated cylinders, beakers, plastic tubes, tube rack, dispenser tips, reagent reservoir for multichannel pipettes, lid, adhesive for microplates, graduated automatic (mono- and multichannel) pipettes, microplate reader, and microplate washer and shaker (optional)

V - PRECAUTIONS FOR USE

- This test may be used for “in vitro” diagnosis only. It is strictly for veterinary use.
- The reagents must be kept between +2°C and +8°C. The reagents cannot be guaranteed if the shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- The concentrated wash solution and dilution buffer may be stored at room temperature. Once diluted, these solutions remain stable for six weeks if kept between +2°C and +8°C.
- Unused strips must be stored immediately in the aluminium envelope, taking care to keep the desiccant dry and the envelope's seal airtight. If these precautions are taken, the strips' activity can be conserved up to the kit's shelf-life date.
- Do not use reagents from other kits.
- The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.
- Discard all solutions contaminated with bacteria or fungi.
- The stop solution contains 1 M phosphoric acid. Handle it carefully.
- All materials and disposable equipment that come in contact with the samples must be considered potentially infectious and be disposed of in compliance with the legislation in force in the country.
- To guarantee the reliability of the results, one must follow the protocol to the letter. Special care must be taken in observing the incubation times and temperatures, as well as measuring the volumes and dilutions accurately.

VI – PROCEDURE

- 1- Bring all components to 21°C +/- 3°C before use. Remove the microplate from its wrapper.
- 2- For blood serum samples, place 1 ml dilution buffer, prepared as instructed in the « Composition of the Kit » section, in 5- or 10-ml hemolysis tubes. Add 10 µl of the serum samples to each of these tubes and shake briefly on a mechanical agitator
- 3- Prepare milks in the following way : centrifuge for 20 minutes at 4000 g. Through the superior layer of the cream, using a glass Pasteur pipette, collect the intermediate liquid, making sure you don't touch the

underlying cellular pellet. Dilute skimmed milks to ¼ in the dilution solution (1 part of milk + 3 parts of dilution solution).

- 4- Dilute the positive serum of the kit to 1/100.
- 5- Distribute 100 µl of the samples per well, respecting the following pattern : positive serum : wells A1 and A2, sample 1 : wells B1 and B2, sample 2 : wells C1 and C2 etc...
- Incubate the plate at 21°C +/- 3°C for one hour. Cover the plate with a lid.
- 6- Rinse the plate with the washing solution, prepared as instructed in the "Composition of the Kit" section, as follows: empty the microplate of its contents by flipping it over sharply above a sink. Tap the microplate upside down against a piece of clean absorbent paper to remove all the liquid. Fill the used wells with the washing solution using a squeeze bottle or by plunging the plate in a vessel of the right dimensions, then empty the wells once more by turning the plate over above a sink. Repeat the entire operation two more times, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times go on to the next step. An automatic plate washer may also be used, but in this case particular care must be taken to avoid any contact between the needles and the bottom of the wells to prevent any damage of the reagent layer.
- 7- Dilute the conjugate 1:50 in the dilution buffer (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.250 ml of diluent). Add 100 µl of the dilute conjugate solution to each well. Incubate for 1 hour at 21°C +/- 3°C. Cover the plate with a lid.
- 8- Wash the plate as described in step 6 above.
- 9- Add 100 µl of the chromogen solution to each well on the plate. The chromogen solution must be absolutely colourless when it is pipetted into the wells. If a blue colour is visible, this means that the solution in the pipette has been contaminated.
- 10- Incubate for 10 minutes at 21°C +/- 3°C protected from the light and uncovered. This time is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
- 11- Add 50 µl of stop solution per microwell.
- 12- Read the optical densities in the microwells using a plate reader and a 450 nm filtre. Results must be read fairly soon after the stopping solution has been added since the chromogen may crystallize in wells with strong signals and thereby distort the data.

VII – INTERPRETING THE RESULTS

Subtract from each value recorded for the odd columns the signal of the corresponding negative control well and write down the result. In performing this calculation, allow for any negative values that may exist. Carry out the same operations for the column corresponding to the positive control.

The test can be **validated** only if the positive serum yields a difference in optical density at 10 minutes that is greater than the value given in the QC data sheet: (**validation: ...**).

Divide the signal read for each sample well by the corresponding positive control serum signal and multiply this result by 100 to express it as a percentage.

$$\text{Val} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

Using the first table in the quality control procedure, determine each serum's or milk's degree of positivity.

A reliable diagnosis can be made only if frank seroconversion can be documented using two coupled serum samples taken at 2- to 3-week intervals. The first sample must be taken during the acute phase of the infection. A frank seroconversion is considered to have occurred if the signal increases by two orders of magnitude (two plusses; for example, ++ -> ++++ or + -> +++). A sample must be considered positive if it yields a result that is **greater than or equal to one plus sign (+)**.

VIII – ORDERING INFORMATION

Neospora caninum ELISA KIT:

2x48 tests
5x48 tests

BIO K 192/2
BIO K 192/5



Li StarFish distribuisce: