SVANOVIR® *Neospora*-Ab

Neospora caninum **iscom ELISA**
Antibody Test

Contents	**Art. No. 104898**
Microtitre plate Microtitre plates (96 wells) coated with non-infectious *Neospora* antigen (sealed and stored dry)	2 (Strips) 12 x 8
Conjugate Lyophilised (horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies)	2
PBS-Tween Solution 20 x concentrate	1 x 125 mL
Substrate Solution (Tetramethylbenzidine in substrate buffer containing H_2O_2) - STORE IN THE DARK!	1 x 20 mL
Stop Solution Contains sulphuric acid (2M) - CORROSIVE! 	1 x 10 mL
A. Positive Control Serum - Contains preservatives	1 x 0.1 mL
B. Negative Control Serum - Contains preservatives	1 x 0.1 mL

This manual covers the following SVANOVIR® *Neospora*-Ab ELISA kit: Article number 104898

Neospora caninum iscom ELISA
Antibody Test

Name and Application
SVANOVIR® *Neospora*-Ab is an enzyme linked immunosorbent assay (ELISA) for the detection of *Neospora* specific antibodies in bovine serum, plasma and milk samples; individual samples, pools and bulk tank milk.

General information
Neospora caninum is an apicomplexan protozoan parasite, which was first described in dogs with neurological disease. *N. caninum* infection is now recognized to be a major cause of bovine abortion and stillbirth worldwide. The parasite is efficiently transmitted transplacentally from an infected cow to her foetus during pregnancy. This results in abortion, birth of a weak calf, or birth of a clinically healthy but persistently infected calf. The mechanism by which the parasite is transmitted from dam to foetus is unknown, as are the factors, which determine the outcome of infection. Transplacental transmission can occur during consecutive pregnancies and congenitally infected heifers can later transmit the parasite to their own offspring, thus the parasite can persist for a long time in an infected herd without involvement of a definitive host. Cattle can also be infected through ingestion of oocysts shed in the faeces of acutely infected dogs, a definitive host of *N. caninum*.
N. caninum induced abortion can occur throughout pregnancy and may include stillbirth at full time but abortions at 5-7 months of gestation is the most common. Bovine abortions caused by *N. caninum* may show epidemic as well as endemic patterns. Epidemiological data indicate that external or point source infections are the most likely cause of abortion outbreaks, whereas a high level or an increase in the annual abortion rate may be a consequence of predominantly transplacental transmission.

Principle
The kit procedure is based on a solid-phase indirect ELISA. In this procedure, samples are exposed to non-infectious *Neospora* antigen incorporated into iscoms coated wells of microtiter strips. *Neospora* antibodies (if present in the sample) bind to the antigen in the wells. The HRP conjugate added subsequently forms a complex with these *Neospora* antibodies. Unbound material is removed by rinsing before the addition of substrate solution. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate. A positive result is indicated by development of a blue colour. The reaction is stopped by addition of the stop solution; the colour changes to yellow. The result can be read visually or by a microplate photometer, where the optical density (OD) is measured at 450 nm.

Materials needed but not provided

1. Precision pipettes
2. Disposable pipette tips
3. Distilled water, deionised or any similar hight quality water
4. Wash bottle, multichannel pipettor or plate washer
5. Container: 1 to 2 litres for PBS-Tween
6. Microplate photometer, 450 nm filter

Specimen information

Serum:

5 µL of blood serum or plasma is needed for each sample well. Fresh, refrigerated or previously frozen serum or plasma may be tested.

Milk:

50 µL of skim milk is required for each sample well. Fresh, refrigerated or previously frozen milk may be tested. Milk samples must be centrifuged for 15 minutes at 2000 x g to remove the lipid layer, or leave the milk samples until the fat layer is formed on top of the sample. Pipette under the fat layer.

Preparation of reagents

PBS-Tween Buffer:

Dilute the PBS-Tween Solution 20 x concentrate 1/20 in distilled water. Prepare 500 mL per plate by adding 25 mL PBS-Tween solution to 475 mL distilled water and mix thoroughly.

N.B. Please check that there is no crystal precipitation in the bottle. If crystals are seen, please warm and shake well.

Pre-dilution of controls and samples:

For testing, the serum controls and serum samples should be pre-diluted 1/100 in PBS-Tween Buffer (for example 5 µL sample into 495 µL of PBS-Tween buffer).

HRP Conjugate:

Reconstitute the lyophilized HRP Conjugate with 11.5 mL PBS-Tween Buffer. Add the buffer carefully to the bottle. Leave the solution one minute and mix thoroughly on a shaker. Prepare immediately before use. The remaining reconstituted conjugate can be stored at -20°C and thawed and refrozen up to 3 times.

Precautions

1. Carefully read and follow all instructions.
2. Store the kit and all reagents at 2-8°C (36-46°F).
3. All reagents should equilibrate to room temperature 18-25°C (64-77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction manuals from different test kit batches.
6. Care should be taken to prevent contamination of kit components.
7. Do not use test kit beyond date of expiry.
8. Do not eat, drink, or smoke where specimens or kit reagents are handled.
9. Use a separate pipette tip for each sample.
10. Do not pipette by mouth.
11. Include positive and negative controls on each plate or test strip series.
12. Use only distilled, deionised or any similar high quality water for preparation of reagents.
13. When preparing the buffers, etc., measure the required volume.
14. The Stop Solution contains sulphuric acid, which is corrosive.*
15. All unused biological materials should be disposed according to the local, regional and national regulations.

Recommendations!

Strips with broken seal can be stored at 2-8°C (36-46°F) for up to 4 weeks.
Reconstituted conjugate may not be stored in refrigerator.

Procedure

1. All reagents should equilibrate to room temperature 18-25°C (64-77°F) before use.
2. In duplicates, add 100 µL of pre-diluted Positive Control Serum (Reagent A) and Negative Control Serum (Reagent B) respectively, into selected wells.

A. **Serum samples**

Add 100 µL of pre-diluted sample to selected well(s). The samples can be tested in singlicates or in duplicates. However for confirmation purposes it is recommended to run the samples in duplicates.

B. **Milk samples**

Add 50 µL of PBS-Tween Buffer to each well that will be used for milk samples.

Add 50 µL of skimmed milk samples to the selected wells.
The samples can be tested in singlicates or in duplicates. However for confirmation purposes it is recommended to run the samples in duplicates.

3. Seal the plate/strip and shake thoroughly. Incubate for 1 hour at 37°C (98.6°F).
4. Rinse the plate/strip 3 times with PBS-Tween Buffer: at each rinse cycle fill up the wells, empty the plate and tap hard to remove all remains of fluid.
5. Add 100 µL HRP Conjugate to each well. Seal the plate/strip and incubate for 1 hour at 37°C (98.6°F).
6. Repeat step #4.
7. Add 100 µL Substrate Solution to each well. Incubate for 10 minutes at room temperature 18-25°C (64-77°F). Begin timing when the first well is filled.
8. Stop the reaction by adding 50 µL of Stop Solution to each well and mix thoroughly. Add the Stop Solution in the same order as the Substrate Solution in step #7.
9. Measure the optical density (OD) of the controls and samples at 450 nm in a microplate photometer (use air as blank). Measure the OD within 15 minutes after the addition of Stop Solution to prevent fluctuation in OD values.

Calculations

Calculation of Percent Positivity Values (PP)

Calculate the mean OD values for the controls and samples.
All OD values for the test samples as well as the Negative Control (Reagent B) are related to the OD value of the positive control as follows:

$$PP = \frac{OD_{\text{Samples or Negative Control}}}{OD_{\text{Positive Control}}} \times 100$$

Interpretation of the results

Criteria for test validity

To ensure validity, the duplicate OD values of the positive control should not differ more than 25 % from the mean value of the two duplicates. Additionally, the control values should fall within the following limits:

OD Positive control	**> 0.8**
OD Negative control	**< 0.15**

For invalid tests, technique may be suspect and the assay should be repeated.

Interpretation of serum and milk samples

PP	Interpretation
< 20	**Negative**
≥ 20	**Positive**

References

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8. Varcasia, A., Capelli, G.., Ruiu, A., Ladu, M., Scala, A., Björkman, C. (2005). Prevalence of *Neospora caninum* infection in Sardinian dairy farms (Italy) detected by iscom ELISA on tank bulk milk. Parasitol. Res. DOI 10.1007/s00436-005-0044-4
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***WARNING: Stop solution (sulphuric acid)**
May be corrosive to metals. Causes skin irritation. Causes serious eye irritation.

Keep only in original container. Wear eye protection/ face protection. Wear protective gloves.
IN CASE OF CONTACT WITH EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician. If eye irritation persists: Get medical advice/ attention.
IN CASE OF CONTACT WITH SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse. If skin irritation occurs: Get medical advice/attention. Absorb spillage to prevent material damage.

Symbols

REF	Article No.
LOT	Serial (batch) No.
	Temperature limit
	Expiry date
	Number of tests
	See manual
	Manufacturer
	Telephone
	Fax


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