General Information

This kit is used to detect anti-*Toxoplasma gondii* antibodies in ruminant, dog, cat or pig sera, plasma or meat juice. Please contact IDvet for use in other species. This indirect ELISA uses the P30 antigen of *Toxoplasma gondii*.

Description and principle

Microwells are coated with the P30 antigen of *Toxoplasma gondii*.

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

After washing, a multi-species peroxidase (HRP) conjugate is added to the wells. It fixes to the antibodies, forming an antigen-antibody-conjugate-HRP complex.

After elimination of the excess conjugate by washing, 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 *p30* antigen
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 2
Dilution Buffer 3
Concentrated Wash Solution (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. 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. All single-use material used for the assays should be decontaminated by immersion in freshly prepared 5% sodium hypochlorite for minimum 1 hour before elimination, or by autoclaving at 120°C.

Sample 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 **(20X)** is completely solubilized.

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

Testing Procedure

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

For sera and plasma – dilution at 1/10 :

1. Add :
   - 90 µl of **Dilution Buffer 2** to each microwell.
   - 10 µl of the **Negative Control** to wells A1 and B1.
   - 10 µl of the **Positive Control** to wells C1 and D1.
   - 10 µl of each sample to be tested to the remaining wells.

For meat juice – dilution at 1/2:

1. Add:
   - 90 µl of **Dilution Buffer 2** and 10 µl of the **Negative Control** to wells A1 and B1.
   - 90 µl of **Dilution Buffer 2** and 10 µl of the **Positive Control** to wells C1 and D1.
   - 50 µl **of Dilution Buffer 2** and 50 µl of each sample to be tested to the remaining wells.

For all applications:

2. Incubate 45 min ± 4 min at 21°C (± 5°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.
4. Prepare the **Conjugate 1X** by diluting the Concentrated **Conjugate 10X** to 1/10 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 O.D. 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%):

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

Samples (sera, plasma or meat juice) presenting a S/P%:

- less than or equal to 40% are considered negative.
- between 40% and 50% are considered doubtful.
- greater than or equal to 50% are considered positive.

Result	Status
S/P % ≤ 40%	NEGATIVE
40% < S/P % < 50%	DOUBTFUL
S/P % ≥ 50%	POSITIVE

Nota: For canine sera, IDvet validation studies (report available upon request) showed that ELISA results between 40% and 70% were difficult to interpret by IFAT. IDvet therefore recommends a wider doubtful zone (40-70%) for canine sera.

IDvet
Innovative Diagnostics

Certified management system

ID Screen®
Toxoplasmosis Indirect Multi-species

Kit for the detection of antibodies directed against *Toxoplasma gondii* by indirect ELISA in sera, plasma and meat juice.

For *in vitro* use

October 2013: Note about canine sera interpretation

TOXOS-MS ver 1014 GB

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