

August 2015

pigtype[®] Toxoplasma Ab Handbook



1 (catalog no. 273401)



5 (catalog no. 273403)



20 (catalog no. 273405)*

For the detection of antibodies to
Toxoplasma gondii

Licensed in accordance with § 17c of the German Law on Animal Diseases
(Flu-B 564)

REF

273401, 273403, 273405*



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* Available only on request.

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Kit Contents

<i>pigtype</i> Toxoplasma Ab	(1)	(5)	(20)
Catalog no.	273401	273403	273405*
Number of plates	1	5	20
Test Plate: microtiter plate with 96 wells, coated with non-infectious <i>Toxoplasma</i> -antigen	1	5	20
Sample Diluent, ready-to-use	1 x 60 ml	2 x 125 ml	2 x 500 ml
Negative Control, ready-to-use	1 x 1.5 ml	1 x 3.5 ml	2 x 3.5 ml
Positive Control, ready-to-use	1 x 1.5 ml	1 x 3.5 ml	2 x 3.5 ml
Wash Buffer (10x)	1 x 125 ml	2 x 125 ml	2 x 500 ml
Conjugate, ready-to-use	1 x 12 ml	1 x 60 ml	1 x 240 ml
TMB substrate, ready-to-use	1 x 12 ml	1 x 60 ml	1 x 240 ml
Stop solution, ready-to-use	1 x 12 ml	1 x 60 ml	1 x 240 ml
Handbook	1	1	1

* Available only on request.

Intended Use

The *pigtype* Toxoplasma Ab is a specific and sensitive ELISA for detecting antibodies to *Toxoplasma gondii* in serum, plasma, and meat juice samples from swine and wild boar (porcine) and in serum and plasma samples from bovine, ovine, caprine, feline, and canine (these include cattle, sheep, goat, cat, dog, and fox). The test kit may also be used for other mammalian species.

The kit is approved by the Friedrich-Loeffler-Institut and registered in accordance with § 17c of the German Law on Animal Diseases (FLI-B 564) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols



<N>

Contains reagents for <N> plates



Legal manufacturer



Lot number



Use by date



Temperature limitations for storage



Handbook



Catalog number



Material number



Protect from light



For pig, cattle, goat, sheep, cat, and dog samples

Storage

The components of the *pigtype* Toxoplasma Ab ELISA should be stored at 2–8°C and are stable until the expiration date stated on

the label. Wash Buffer (10x) and Stop Solution may be stored at room temperature (18–25°C) to avoid salt crystallization. If test strips are provided with the kit, store the remaining test strips in the re-sealed foil pouch with desiccant at 2–8°C until next use. The test strips can be stored for at least 6 weeks after opening the plate pouch.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view and print the SDS for each QIAGEN kit and kit component.



CAUTION: The Stop Solution contains 0.5 M sulfuric acid.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *pigtype* Toxoplasma Ab is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *pigtype* *Toxoplasma* Ab is a highly sensitive and specific solution for the detection of antibodies to *Toxoplasma gondii*. The kit permits a quick and reliable detection of antibodies to *Toxoplasma gondii* in serum, plasma, and meat juice samples from swine and wild boar (porcine) and in serum and plasma samples from bovine, ovine, caprine, feline, and canine (these include cattle, sheep, goat, cat, dog, and fox).

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii*, which is spread worldwide in warm-blooded animals, including humans. Cats and other Felidae play an important role for epidemiology of toxoplasmosis since they are the definitive host and therefore shedders of *Toxoplasma gondii*-oocysts. Primary infection of humans occurs by ingestion of sporulating oocysts from feces of recently infected cats. Another source of infection can be raw or undercooked pork, containing *Toxoplasma*-oocysts.

Principle

The microtiter test plate is coated with *Toxoplasma gondii* tachyzoite-antigen. During sample incubation *Toxoplasma*-specific antibodies bind to the immobilized antigen. Unbound material is removed by rinsing. The multi-species horseradish peroxidase (HRP) conjugate detects serum antibodies bound to the antigen. Unbound conjugate is removed by rinsing. A colorimetric reaction is initiated by adding Substrate Solution and stopped after 10 minutes. In the presence of *Toxoplasma*-specific antibodies, within

the sample, HRP catalyzes a blue color development, which turns yellow after adding the Stop Solution. The optical density (OD) is measured in a spectrophotometer. The OD values correlate with the concentration of anti-*Toxoplasma* antibodies in the sample.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

All sample types

- Beakers
- Measuring cylinders
- Pipets (adjustable)
- Multichannel pipets (adjustable)
- Aluminum or adhesive foil for covering the Test Plate
- Optional: Device for delivery and aspiration of wash solution
- Microtiter plate absorbance reader
- Tubes or plates for diluting the samples
- Distilled water

Meat juice samples

- Meat juice sampling device

Important Notes

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight when performing the test.
- Components of the test kit should not be contaminated or mixed with components from other batches.
- Do not use the components of the test kit past the expiration date.
- Water from ion-exchange systems used for diluting the Wash Buffer (10x) may interfere with the assay if not pure enough. Use double-distilled water or highly purified water (e.g. Milli-Q®).
- The use of clean glass devices, careful pipetting and rinsing during the test, and strict adherence to the indicated incubation times is essential for precise test results.

Preparation of meat juice

To extract meat juice samples, freeze approximately 10 g blood- and fat-free muscle meat (e.g., from the muscular pillars of lumbar diaphragm) in a meat juice sampling device, and then thaw. Take the meat juice released from the thawed samples and store at 2–8°C. Samples stored at 2–8°C should be analyzed within 24 hours. Alternatively, meat juice samples can be stored at –20°C for several months until required for analysis.

Protocol: ELISA Test Procedure

Important points before starting

- Please read “Important Notes” on page 10 before starting.
- Dilute serum and plasma samples prior to analysis.
- Meat juice samples can be diluted prior to analysis or can be diluted directly in the Test Plate.
- Controls are ready to use and do not require dilution.

Things to do before starting

- Bring reagents to room temperature (15–25°C) just before use. In case of precipitated salt crystals in the Wash Buffer (10x), dissolve by gentle swirling and warming.
- Dilute Wash Buffer (10x) 1:10 in distilled water. For example, for one Test Plate dilute 25 ml Wash Buffer (10x) in 225 ml distilled water and mix.
- Dilute serum and plasma samples 1:100 in Sample Diluent prior to analysis (e.g., dilute 5 µl sample in 495 µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution. Use a fresh pipet tip for each sample.
- If required, meat juice samples can be diluted prior to analysis. Dilute meat juice samples 1:10 in Sample Diluent (e.g., dilute 25 µl sample in 225 µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution. Use a fresh pipet tip for each sample.

Procedure

1. If using samples that were diluted prior to analysis, go to step 1a. If samples should be diluted in the Test Plate, go to step 1b.

1a. Pipet 100 μ l each of the Negative Control (in duplicates), Positive Control (in duplicates) and the diluted serum, plasma or meat juice samples into the wells of the Test Plate. Proceed to step 2.

Note: Record the positions of the controls and samples in a test protocol. We recommend use of a multichannel pipet for sample transfer. Cover the Test Plate.

1b. Pipet 100 μ l each of the Negative Control (in duplicates) and Positive Control (in duplicates), into the wells of the Test Plate. Dispense 90 μ l Sample Diluent into each sample well of the Test Plate and add 10 μ l undiluted meat juice. Mix well. Proceed to step 2.

Note: Record the positions of the controls and samples in a test protocol. Mix either by using a plate shaker or by repeated pipetting up and down. Cover the Test Plate.

2. Incubate for 60 min at room temperature (15–25°C) or overnight (12–18 hours) at 2–8°C.
3. Remove solution from the wells by aspiration or tapping.
4. Rinse each well 3x with 300 μ l of prepared Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
5. Pipet 100 μ l ready-to-use Conjugate into each well and incubate for 30 min at room temperature.
6. Remove solution from wells by aspiration or tapping.

-
7. Rinse each well 3x with 300 μ l of prepared Wash Buffer.
Remove the buffer after each rinse by aspiration or tapping.
 8. Pipet 100 μ l TMB Substrate Solution into each well.
 9. Incubate for 10 min at room temperature in the dark. Begin timing after the first well is filled.
 10. Stop the reaction by adding 100 μ l Stop Solution per well.
Add the Stop Solution in the same order as the Substrate Solution was added.
 11. Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.
Measurement at a reference wavelength (620–650 nm) is optional.

Data Interpretation

Validation criteria

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 0.7 .
- The MV of the measured OD value for the Negative Control (NC) must be ≤ 0.25 using the short protocol (incubation for 60 min at room temperature) or ≤ 0.5 using the overnight protocol (incubation for 12-18 hours at 2-8°C).

In case of invalid assays, the test should be repeated after carefully reading the instructions for use.

Calculation

Calculate the MV of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the Positive Control is calculated according to the following equation:

$$S/P = \frac{OD_{\text{sample}} - MV OD_{\text{NC}}}{MV OD_{\text{PC}} - MV OD_{\text{NC}}}$$

Interpretation of the results

For samples from pig, sheep, goat, fox, dog and cat

Short protocol (incubation for 60 min at room temperature)

- Samples with S/P ratio ≥ 0.3 are positive.
Specific antibodies to *Toxoplasma gondii* were detected.
- Samples with S/P ratio < 0.3 are negative.
Specific antibodies to *Toxoplasma gondii* could not be detected.

Overnight protocol (incubation at 2-8°C for 12-18 h)

- Samples with S/P ratio ≥ 0.5 are positive.
Specific antibodies to *Toxoplasma gondii* were detected.
- Samples with S/P ratio < 0.5 are negative.
Specific antibodies to *Toxoplasma gondii* could not be detected.

For samples from wild boar and cattle

Short protocol (incubation for 60 min at room temperature)

- Samples with S/P ratio ≥ 0.2 are positive.
Specific antibodies to *Toxoplasma gondii* were detected.
- Samples with S/P ratio < 0.2 are negative.
Specific antibodies to *Toxoplasma gondii* could not be detected.

Overnight protocol (incubation at 2-8°C for 12-18 h)

- Samples with S/P ratio ≥ 0.4 are positive.
Specific antibodies to *Toxoplasma gondii* were detected.
- Samples with S/P ratio < 0.4 are negative.
Specific antibodies to *Toxoplasma gondii* could not be detected.

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit www.qiagen.com).

Appendix: Quick Guide

Sample dilution: Serum/plasma 1:100 and meat juice 1:10, mix well.

Step	Short protocol	Overnight protocol
1. Sample		100 µl/well
2. Incubation	60 min RT	12–18 h, 2–8°C
3. Wash		3 x 300 µl
4. Conjugate		100 µl/well
5. Incubation		30 min RT
6. Wash		3 x 300 µl
7. TMB		100 µl/well
8. Incubation		10 min RT
9. Stop		100 µl/well
10. Read		450 nm

Data interpretation

For samples from pig, sheep, goat, fox, dog and cat

Sample	Negative	Positive
Short protocol	S/P <0.3	S/P ≥0.3
Overnight protocol	S/P <0.5	S/P ≥0.5

For samples from wild boar and cattle

Sample	Negative	Positive
Short protocol	S/P <0.2	S/P ≥0.2
Overnight protocol	S/P <0.4	S/P ≥0.4

Ordering Information

Product	Contents	Cat. no.
<i>pigtype</i> Toxoplasma Ab (1)	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273401
<i>pigtype</i> Toxoplasma Ab (5)	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273403
<i>pigtype</i> Toxoplasma Ab (20)*	For 1920 reactions: 20 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273405
Related products		
<i>pigtype</i> CSFV E ^{ns} Ab (5) [†]	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, HRP Conjugate, TMB Substrate Solution, Stop Solution	272303
<i>pigtype</i> PRRSV Ab (5) [†]	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	272753

* Available only on request. † Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
<i>pigtype</i> PRRSV OF Ab (1)	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	272771
<i>pigtype</i> Salmonella Ab (5)*	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273003
<i>pigtype</i> HEV Ab (1)	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	272501
<i>pigtype</i> Trichinella Ab (1)	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273501
<i>pigtype</i> Yersinia Ab (1)	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273801

* Other kit sizes are available; see www.qiagen.com.

QIAGEN offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens. Visit **www.qiagen.com/Animal-and-Veterinary-Testing** for more information about *bactotype*[®], *cador*[®], *cattletype*[®], *flocktype*[®], *pigtype* and *virotype*[®] products.

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