January 2015

pigtype® Trichinella Ab Handbook

1 (catalog no. 273501)

5 (catalog no. 273503)

20 (catalog no. 273505)*

Multi-species ELISA kit for the detection of antibodies to *Trichinella* spp.

REF 273501, 273503, 273505*

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* Available only on request.

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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In addition, QIAGEN provides high-quality, easy-to-use, and sensitive molecular solutions to enable veterinary pathogen detection and animal pathogen research. The QIAGEN veterinary portfolio includes a broad range of pathogen-specific PCR-assays and an extensive and growing ELISA portfolio. For more information, visit www.qiagen.com/Animal-and-Veterinary-Testing.

Contents

Kit Contents

pigtype **Trichinella Ab**			
Catalog no.	**273501**	**273503**	**273505***
Number of plates	**1**	**5**	**20**
Test Plate: microtiter plate with 96 wells, coated with non-infectious *Trichinella* E/S-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* Trichinella Ab is an indirect enzyme immunoassay (ELISA). It is intended for the detection of antibodies to *Trichinella* spp. in pig and wild boar serum, plasma, and meat juice samples. Pig and wild boar serum and plasma samples can be tested as pools of up to 10 individual samples. The test kit may also be used for other mammalian species e.g., horse, fox, and rodents. For veterinary use only.

Symbols

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 and wild boar samples

Storage

The components of the *pigtype* Trichinella 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. Store the remaining unused 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 sulphuric acid.

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

Quality Control

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

Introduction

The *pigtype* Trichinella Ab is a highly sensitive solution for the detection of antibodies to *Trichinella* spp. Trichinellosis is a zoonosis which is caused by the nematode *Trichinella*. In humans the infection can cause mild to lethal illness. Humans are infected by consumption of pork containing *Trichinella*-larvae. Most infected pigs do not show clinical symptoms. To prevent human infections, all pigs slaughtered for the food chain are tested by artificial digestion.

The European Commission Regulation (EC) No. 2075/2005 allows holdings to be certified as *Trichinella*-free under certain conditions. One of these conditions is a monitoring program. Serological testing can provide a more cost-effective method, to the commonly used digestion-method, for the monitoring of *Trichinella* antibodies in pork from integrated production systems. The *pigtype* Trichinella Ab can be used for monitoring *Trichinella* infections as part of such programs.

Principle

pigtype Trichinella Ab is an indirect ELISA. The microtiter plate is coated with inactivated *Trichinella*-antigen (E/S-antigen). During sample incubation *Trichinella*-specific antibodies bind to the immobilized antigen. Unbound material is removed by rinsing. Serum antibodies bound to the antigen are detected by a multi-species horseradish peroxidase (HRP) conjugate. Unbound conjugate is removed by rinsing. A colorimetric reaction is initiated by adding Substrate Solution and stopped after 10 minutes. In the presence of *Trichinella*-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 using a spectrophotometer at 450 nm. The OD values correlate with the concentration of *Trichinella*-specific 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.

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

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight during the performance of 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 expiration date.
- Water from ion-exchange systems used for diluting the Wash Solution (10x) may interfere with the assay if not pure enough. Water quality of double distilled water or highly purified water (Milli-Q) is suitable.
- 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.

Things to do before starting

- Bring reagents to room temperature (18–25°C) immediately before use. In case of precipitated salt crystals in the Wash Buffer (10x), dissolve by gentle swirling and warming.

Wash Buffer: Dilute Wash Buffer (10x) 1:10 in distilled water, e.g., for one test plate dilute 25 ml Wash Buffer (10x) in 225 ml distilled water and mix.

Serum/plasma: Prior to sample analysis, with serum/plasma samples, dilute **1:100** in Sample Diluent (e.g., dilute 5 µl sample in 495 µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution. Change pipet tips for each sample.

Pool samples: Serum or plasma samples can also be tested as pools of 10 single samples (e.g., pool 10 µl of each of 10 samples). Dilute the pool samples **1:20** with Sample Diluent (e.g., dilute 10 µl sample in 190 µl Sample Diluent) and mix.

Meat juice: Prior to sample analysis, with meat juice samples, dilute **1:10** in Sample Diluent (e.g., dilute 25 µl sample in 225 µl Sample Diluent) and mix well.

Alternatively, meat juice samples can be diluted directly in the Test Plate (see Procedure 1a).

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 analysis).

- **Controls** are ready-to-use and do not require dilution.

Protocol: ELISA

Please read "Things to do before starting", page 11.

Procedure

1. Pipet 100 µl of each of the ready-to-use Negative Control (in duplicates) and Positive Control (in duplicates) and the 1:10 diluted meat juice samples and/or 1:100 diluted serum or plasma samples into the Test Plate wells.

1a. Alternatively, pipet 90 µl of Sample Diluent in each sample well and add 10 µl of the undiluted meat juice sample and/or of the 1:10 pre-diluted serum or plasma sample. Mix well.

   Record the positions of the controls and samples in a test protocol. The use of a multichannel pipet is recommended for the transfer of samples. Cover the Test Plate.

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

    Measuring 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 OD of the Positive Control must be ≥0.7
- The mean OD of the Negative Control must be ≤0.2

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation

Calculate the mean values (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:

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

Interpretation short protocol (60 min sample incubation)

Samples with S/P-ratio ≥0.3 are positive.
Specific antibodies to *Trichinella* spp. were detected.

Samples with S/P-ratio <0.3 are negative.
Specific antibodies to *Trichinella* spp. could not be detected.

Interpretation overnight protocol (O/N sample incubation)

Samples with S/P-ratio ≥0.45 are positive.
Specific antibodies to *Trichinella* spp. were detected.

Samples with S/P-ratio <0.45 are negative.
Specific antibodies to *Trichinella* spp. 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, see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
pigtype 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
pigtype Trichinella Ab (5)	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273503
pigtype Trichinella Ab (20)*	For 1920 reactions: 20 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273505
Related products		
pigtype 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
pigtype 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

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

Product	Contents	Cat. no.
pigtype 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

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

QIAGEN offer 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 the *bactotype*®, *cador* ®, *cattletype*®, *flocktype*®, *pigtype*, and *virotype*® products.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Quick guide

Sample dilution:
Serum/plasma 1:100 Meat juice 1:10

Step	Short protocol	Overnight protocol
1. Sample	100 µl/well	
2. Incubation	60 min. RT	Overnight 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

	Negative	Positive
Short protocol	S/P <0.3	S/P ≥0.3
Overnight protocol	S/P <0.45	S/P ≥0.45

Notes

Notes

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