August 2018 | EN

pigtype® Trichinella Ab Handbook

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

REF 1 plate (cat. no. PT273501)

INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

INDICAL BIOSCIENCE

Contents

Kit contents

pigtype Trichinella Ab **Cat. no.** **Number of plates**	**(1)** **PT273501** **1**
Test Plate: microtiter plate with 96 wells, coated with non-infectious *Trichinella* E/S-antigen	1
Sample Diluent, ready to use	1 x 60 ml
Negative Control, ready to use	1 x 1.5 ml
Positive Control, ready to use	1 x 1.5 ml
Wash Buffer, 10x concentrate	1 x 125 ml
Conjugate, ready to use	1 x 12 ml
TMB Substrate, ready to use	1 x 12 ml
Stop Solution, ready to use	1 x 12 ml
Handbook	1

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

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

 For samples from pig and wild boar

Quality control

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

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. 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 from your local sales representative or by Email request under **compliance@indical.com**.

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.

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

The 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
- Optional: Device for delivery and aspiration of Wash Buffer
- Microtiter plate absorbance reader
- Tubes or plates for diluting the samples
- Distilled water

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 (Milli-Q®).
- For accurate test results, it is essential to use clean glassware and to pipet and rinse carefully and strictly adhere to the incubation times when performing the test.

Protocol: ELISA test procedure

Important points before starting

- Please read „Important notes“ on page 8 before starting.
- Dilute serum and plasma samples before analysis.
- Optional: pooling and pre-dilution.
- Controls are ready to use and do not require dilution.

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.
- 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.
- Serum/ plasma samples: 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. Dispense 90 µl Sample Diluent into each well. Add 10 µl of undiluted meat juice sample and mix well (see procedure 1b).

Extract meat juice samples from approximately 10 g non-fat non-blood contaminated tissue, for example, from the diaphragm pillar, in a meat juice sampling device by freezing and thawing. 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).

Protocol: ELISA

Please read „Things to do before starting“, page 9.

Procedure

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

1a. Pipet 100 µl of each of the ready to use Negative Control (in duplicates), Positive Control (in duplicates), and the 1:10 diluted meat juice samples, 1:20 diluted pool samples, and/ or 1:100 diluted serum or plasma samples into the Test Plate wells. Proceed to step 2.

Note: 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.

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

Note: Record the positions of the controls and samples in a test protocol. Mix by either using a plate shaker or by repeated liquid aspirating and dispensing. 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 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
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 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
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 value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 0.7.
- The mean value (MV) of the measured OD value for the Negative Control (NC) must be ≤ 0.2.

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 ration (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 of the results

Short protocol (60 min sample incubation at RT)

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

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

- **Samples with S/P-ratio ≥ 0.45 are positive**.
  Specific antibodies to *Trichinella* were detected.
- **Samples with S/P-ratio < 0.45 are negative**.
  Specific antibodies to *Trichinella* could not be detected.

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.

Visit **www.indical.com** for more information about bactotype, cador, cattletype, flocktype, pigtype and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL kit handbook or user manual.

Limited License Agreement for pigtype Trichinella Ab

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. INDICAL grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.indical.com. Some of these additional protocols have been provided by INDICAL users for INDICAL users. These protocols have not been thoroughly tested or optimized by INDICAL. INDICAL neither guarantees them nor warrants that they do not infringe the rights of third-parties.

2. Other than expressly stated licenses, INDICAL makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.

3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

4. INDICAL specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. INDICAL may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.indical.com.

Change index

Handbook	Version	Change
HB-1576-EN-004	August 2018	INDICAL design

Quick guide for pigtype Trichinella Ab

Sample dilution:

Serum, plasma 1:100, meat juice 1:10; mix well

Step	Short protocol	Overnight protocol
1. Sample	100 µl/ well	
2. Incubation	60 min at RT	Overnight (12-18h) at 2-8°C
3. Wash	3 x 300 µl	
4. Conjugate	100 µl/ well	
5. Incubation	30 min at RT	
6. Wash	3 x 300 µl	
7. TMB	100 µl/ well	
8. Incubation	10 min at 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

INDICAL BIOSCIENCE

Ordering: **www.indical.com/contact**
Technical Support: **support@indical.com**
Website: **www.indical.com**