

pigtype[®] Yersinia Ab Handbook



1 (catalog no. 273801)



5 (catalog no. 273803)*



20 (catalog no. 273805)*

For the detection of antibodies to
pathogenic *Yersiniae*

REF

273801, 273803, 273805



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* Available only on request.

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- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Kit Contents

<i>pigtype</i> Yersinia Ab			
Catalog no.	273801	273803*	273805*
Number of plates	1	5	20
Test Plate: microtiter plate with 96 wells, coated with non-infectious <i>Yersinia</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* Yersinia Ab is a specific and sensitive ELISA for detecting antibodies to pathogenic *Yersiniae* in porcine serum, plasma, and meat juice samples. 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 samples

Storage

The components of the *pigtype* Yersinia 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 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* Yersinia Ab is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *pigtype* Yersinia Ab is a highly sensitive and specific solution for the detection of antibodies to pathogenic *Yersinia* strains. The antigens (*Yersinia* Outer Proteins) used in this test are produced by pathogenic strains only, spanning all serotypes. Therefore, antibody cross reactivity to non-pathogenic species or other enterobacteria can be excluded. Fifteen species belong to the genus *Yersinia*. Three of them (*Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis*) are pathogens for humans. Following directive 2003/99/EG yersiniosis, and the agents thereof, are zoonoses to be monitored.

pigtype Yersinia Ab is an effective method of monitoring the infection status of herds over extended periods and of controlling the serological status of purchased animals.

Principle

The *pigtype* Ab is an indirect ELISA. The microtiter test plate is coated with non-infectious, recombinant *Yersinia* antigen. During sample incubation *Yersinia*-specific antibodies bind to the immobilized antigen. Unbound material is removed by rinsing. The anti-IgG-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 *Yersinia*-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-*Yersinia* 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, for example, 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 μ l sample in 495 μ l Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution. Change pipet tips for each sample.

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

Alternatively, meat juice samples can be diluted directly in the Test Plate. Dispense 90 μ l Sample Diluent into each well. Add 10 μ l of undiluted meat juice sample and mix well (see procedure 1a).

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).

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

Protocol: ELISA

Please read “Things to do before starting”, page 10.

Procedure

1. Pipet 100 μ l of each of the ready-to-use Negative Control (in duplicates), 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 μ l of Sample Diluent in each sample well and add 10 μ l of the undiluted meat juice 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 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.
5. Pipet 100 μ l 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 μ l of prepared Wash Buffer. Remove the buffer after each rinse.
8. Pipet 100 μ l 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 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.

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 MV of the measured OD value for the Negative Control (NC) must be ≤ 0.3

In case of invalid assays the test should be repeated after a thorough review of 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

Short protocol (60 min sample incubation)

Samples with S/P-ratio ≥ 0.3 are positive.

Specific antibodies to *Yersinia* spp. were detected.

Samples with S/P-ratio < 0.3 are negative.

Specific antibodies to *Yersinia* spp. could not be detected.

Overnight protocol (O/N sample incubation)

Samples with S/P-ratio ≥ 0.4 are positive.

Specific antibodies to *Yersinia* spp. were detected.

Samples with S/P-ratio < 0.4 are negative.

Specific antibodies to *Yersinia* 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.
<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
<i>pigtype</i> Yersinia Ab (5)*	For 192 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273803
<i>pigtype</i> Yersinia Ab (20)*	For 96 reactions: 20 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273805
Related products		
<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> 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

* Available only on request.

[†] Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
<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

* 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*[®].

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 <0.4	S/P ≥0.4

Notes

Notes

Notes

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