January 2015

pigtype® Salmonella Ab Handbook

1 (catalog no. 273001)

5 (catalog no. 273003)

20 (catalog no. 273005)*

For the detection of antibodies to salmonella serotypes of group B, C, D, and E

Registered in accordance with § 17c of the German Law on Animal Diseases (BFAV-B 380)

REF 273001, 273003, 273005*

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* Available only on request.

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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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* Salmonella Ab**			
Catalog no.	**273001**	**273003**	**273005***
Number of plates	**1**	**5**	**20**
Test Plate: microtiter plate with 96 wells, coated with non-infectious salmonella-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* Salmonella Ab is a specific and sensitive ELISA for detecting antibodies to salmonella serotypes of group B, C, D, and E in serum, plasma, and meat juice samples from swine. The kit is approved by the Friedrich-Loeffler-Institut and registered in accordance with § 17c of the German Law on Animal Diseases (BFAV-B 380) for use in Germany for veterinary diagnostic procedures. 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* Salmonella 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* Salmonella Ab is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *pigtype* Salmonella Ab is a highly sensitive and specific solution for the detection of antibodies to *Salmonella* spp. Antibodies to the O-antigens 1, 3, 4, 5, 6, 7, 9, 10, and 12 are detected and therefore salmonella serotypes of group B, C, D and E (Kauffmann-White-scheme). The kit can be used to test swine serum, plasma, and/or meat juice samples.

Salmonellosis is a zoonotic disease caused by bacteria of the genus Salmonella. Humans can be infected by consumption of salmonella contaminated raw or undercooked pork. The infection can cause infectious gastroenteritis. *pigtype* Salmonella Ab permits the detection of antibodies to more than 97% of the most frequently occurring salmonella serotypes. The directive of the EU 2003/99/EG, on the monitoring of zoonoses and zoonotic agents, demands efficient surveillance and control programmes regarding prevention and consumer protection. Testing of sera or meat juice samples with the ELISA technique is the most reliable method to define the salmonella status in swine herds. Several countries have introduced monitoring programmes in compliance with the Danish standard to guarantee high quality meat and meat products. In order to German salmonella regulation (BGBI. chapter I, No. 10 2007, p. 322), swine herds can be classified according to their serological results.

Principle

The *pigtype* Salmonella Ab is an indirect ELISA. The microtiter test plate is coated with salmonella-antigen (LPS-antigen). During sample incubation salmonella-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 salmonella-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-salmonella 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 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.

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 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 11.

Procedure

1. 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 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. 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 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 ≤0.2 using the **short protocol**
- The MV of the measured OD value for the Negative Control (NC) must be ≤0.3 using the **overnight protocol**

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:

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

Alternatively, OD% values can be calculated according to the following equation:

$$OD\% = \frac{S/P \times 100\ \%}{2}$$

Interpretation of the results

Short protocol (60 min sample incubation)

Samples with S/P-ratio ≥0.3 (15% OD) are positive.
Specific antibodies to salmonella were detected.

Samples with S/P-ratio <0.3 (15% OD) are negative.
Specific antibodies to salmonella could not be detected.

Overnight protocol (O/N sample incubation)

Samples with S/P-ratio ≥0.4 (20% OD) are positive.
Specific antibodies to salmonella were detected.

Samples with S/P-ratio <0.4 (20% OD) are negative.
Specific antibodies to salmonella could not be detected.

Herd classification in monitoring programs (for both protocols)
National salmonella control programs might consider factors in addition to the scientific cut-off values (e.g., salmonella prevalence). Therefore, in local programs specific cut-off values were defined for diagnostic routine.
For example, the herd screening according to the Danish and German monitoring program implies the following cut-off values:

Samples with S/P < 0.8 (40% OD) are negative.
Samples with S/P ≥ 0.8 (40% OD) are positive.
Other regional monitoring programs may use other cut-off values, which should be applied according to the local regulations and guidelines.

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 Salmonella Ab (1)	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273001
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 Salmonella Ab (20)*	For 1920 reactions: 20 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273005
Related products		
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 Yersinia Ab (5)*	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273803

* 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*®.

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 (15% OD)	S/P ≥0.3 (15% OD)
Overnight protocol	S/P <0.4 (20% OD)	S/P ≥0.4 (20% OD)
Monitoring (both protocols)	S/P <0.8 (40% OD)	S/P ≥0.8 (40% OD)

Notes

Notes

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. QIAGEN 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.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.

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