May 2018

pigtype® Salmonella Ab Handbook

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

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: BFAV-B 380

REF 1 plate (cat. no. PT273001)

REF 5 plates (cat. no. PT273003)

REF 20 plates (cat. no. PT273005)

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INDICAL BIOSCIENCE

Contents

Kit contents

pigtype Salmonella Ab **Cat. no.** **Number of plates**	**(1)** **PT273001** **1**	**(5)** **PT273003** **5**	**(20)** **PT273005** **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 concentrate	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

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-Institute and licensed in accordance with § 11 (2) of the German Animal Health Act (BFAV-B 380) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

 For pig samples

Quality control

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

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 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 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
- 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.

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.
- 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 9.

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.

   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. 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 (18-25°C).
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 using the **short protocol**.
- The mean value (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 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:

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

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.

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.

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Change index

Handbook	Version	Change
HB-1590-006	May 2018	INDICAL design

Quick guide for pigtype Salmonella 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 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 (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%)

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