May 2019 | EN

pigtype® PRRSV Ab Handbook

For the detection of antibodies to *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV)

REF 5 plates (cat. no. PT272753)

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

INDICAL BIOSCIENCE

Contents

Kit contents 3
Intended use 3
Symbols 4
Quality control 4
Storage 4
Safety information 5
Introduction 6
Principle 6
Equipment and reagents to be supplied by user 7
Important notes 8
General precautions 8
Protocol: ELISA test procedure 9
Important points before starting 9
Things to do before starting 9
Protocol: ELISA 10
Data interpretation 12
Validation criteria 12
Calculation 12
Interpretation of the results 13
Change index 15
Quick guide for pigtype PRRSV Ab 16

Kit contents

pigtype PRRSV Ab **Cat. no.** **Number of plates**	**(5)** **PT272753** **5**
Test Plate: microtiter plate with 96 wells, coated with recombinant, inactivated EU- and NA-specific PRRSV antigens	5
Sample Diluent, ready to use	1 x 125 ml
Negative Control, ready to use	2 x 3.5 ml
Positive Control, ready to use	2 x 3.5 ml
Wash Buffer, 10x concentrate	2 x 125 ml
Conjugate, ready to use	1 x 60 ml
TMB Substrate, ready to use	1 x 60 ml
Stop Solution, ready to use	1 x 60 ml
Handbook	1

Intended use

The pigtype PRRSV Ab is a specific and sensitive ELISA for the detection of antibodies to *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV) in serum and plasma samples from pigs.

For veterinary use only.

Symbols

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

 For porcine samples

Quality control

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

Storage

The components of the pigtype PRRSV 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

PRRS virus infections in pigs are highly prevalent and have a detrimental economic impact on the pig industry.

PRRSV is an RNA virus that is a member of the family *Arteriviridae*, order *Nidovirales*. PRRS viruses are classified into the European (EU/I) and the North American (NA/II) genotype. PRRS virus infections can cause respiratory disease in piglets and reproductive failure in pregnant sows.

Serology is widely used as a tool for diagnostic purposes. The pigtype PRRSV Ab is a highly sensitive and specific solution for the detection of antibodies to PRRSV (NA and EU virus types) in serum and plasma samples from pigs. It is, thus, an effective method of monitoring the vaccination or infection status of pig herds.

Principle

The pigtype PPRSV Ab is an indirect ELISA. The microtiter test plate is coated with recombinant PRRSV antigens, specific for NA and EU virus types. During sample incubation, antibodies specific for PRRSV bind to the immobilized antigen. Unbound material is removed by rinsing. Antibodies bound to the antigen are detected by a horseradish peroxidase (HRP) conjugate. Unbound conjugate is removed by rinsing. A colorimetric reaction is initiated by adding Substrate Solution and stopped after 10 minutes. If antibodies specific for PRRSV are present in the sample, a blue color develops, which turns yellow after the addition of Stop Solution. The optical density (OD) is measured in a spectrophotometer at 450 nm. The OD value correlates to the concentration of antibodies specific for the NA/EU strains of PRRSV 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.
- Serum and plasma samples can be diluted prior to analysis or can be diluted directly in the Test Plate.
- 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 50 ml Wash Buffer (10x) in 450 ml distilled water and mix.
- If required, serum and plasma samples can be diluted prior to analysis. Dilute serum or plasma samples **1:40** in Sample Diluent (e.g., dilute 5 µl sample in 195 µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution. Use a fresh pipet tip for each sample.

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 should be diluted in the Test Plate, go to step 1b.

1a. Pipet 100 µl each of the Negative Control (in duplicates), Positive Control (in duplicates) and the **1:40 prediluted** serum or plasma samples into the wells of the Test Plate. Proceed to step 2.

Note: Record the positions of the controls and samples in a test protocol. We recommend use of a multichannel pipet for sample transfer. Cover the Test Plate.

1b. Pipet 100 µl each of the Negative Control (in duplicates), Positive Control (in duplicates) into the wells of the Test Plate. Dispense 97.5 µl of Sample Diluent into each sample well of the Test Plate and add 2.5 µl **undiluted** serum or plasma sample. Mix well. Proceed to step 2.

Note: Record the positions of the controls and samples in a test protocol. Mix either by using a plate shaker or by repeated pipetting up and down. Cover the Test Plate.

2. Incubate for 30 min at room temperature (18-25°C).
3. Remove solution from the wells by aspiration or tapping.
4. Rinse each well 5x 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 into each well and incubate for 30 min at room temperature.
6. Remove solution from wells by aspiration or tapping.
7. Rinse each well 5x 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 into 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 ≥ 0.5.
- The mean value (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 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 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 of the results

- **Samples with S/P-ratio < 0.4 are negative**.
  Specific antibodies to PRRSV could not be detected.
- **Samples with S/P-ratio ≥ 0.4 are positive**.
  Specific antibodies to PRRSV were 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 PRRSV 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-1944-EN-004	May 2019	Increase of volume for assay controls
HB-1944-EN-003	Aug 2018	INDICAL design

Quick guide for pigtype PRRSV Ab

Sample dilution:

Serum, plasma 1:40; mix well

Step	
1. Sample	100 µl/ well
2. Incubation	30 min at RT
3. Wash	5 x 300 µl
4. Conjugate	100 µl/ well
5. Incubation	30 min at RT
6. Wash	5 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

Sample	Negative	Positive
Serum, plasma	S/P < 0.4	S/P ≥ 0.4

INDICAL BIOSCIENCE

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