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pigtype® PRRSV Ab Handbook

Σ 1 (catalog no. 272751)*

Σ 5 (catalog no. 272753)

Σ 20 (catalog no. 272755)*

For detection of antibodies to porcine reproductive and respiratory syndrome virus (PRRSV) in serum or plasma

REF 272751,* 272753, 272755*

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* Available only on request.

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Kit Contents

***pigtype* PRRSV Ab**	**(1)**	**(5)**	**(20)**
Catalog no.	**272751***	**272753**	**272755***
Number of plates	**1**	**5**	**20**
Test Plate: microtiter plate with 96 wells, coated with recombinant, inactivated EU- and NA-specific PRRSV antigens	1	5	20
Sample Diluent, ready to use	1 x 60 ml	1 x 125 ml	2 x 125 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* PRRSV Ab is a specific and sensitive ELISA for detection of antibodies to porcine reproductive and respiratory syndrome virus (PRRSV) in serum and plasma samples from pigs. For veterinary use only.

Symbols

 <N>	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 porcine samples

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 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 sulfuric acid.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Quality Control

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

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 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 (e.g. 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 10 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.

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 dilutions of 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 Sample Diluent into each sample well of the Test Plate and add 2.5 µl undiluted 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 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 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.

    Measurement 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 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 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 positive.**
  Specific antibodies to PRRSV were detected.
- **Samples with S/P ratio <0.4 are negative.**
  Specific antibodies to PRRSV 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, visit **www.qiagen.com**).

Appendix: Quick Guide

Sample dilution: Serum/plasma 1:40

Step	
1. Sample	100 µl/well
2. Incubation	30 min at room temperature
3. Wash	5 x 300 µl
4. Conjugate	100 µl/well
5. Incubation	30 min at room temperature
6. Wash	5 x 300 µl
7. TMB	100 µl/well
8. Incubation	10 min at room temperature
9. Stop	100 µl/well
10. Read	450 nm

Data interpretation

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

Ordering Information

Product	Contents	Cat. no.
pigtype PRRSV Ab (1)*	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	272751
pigtype PRRSV Ab (5)	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	272753
pigtype PRRSV Ab (20)*	For 1920 reactions: 20 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	272755
Related products		
pigtype PRRSV OF Ab (1)†	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	272771
virotype® PRRSV RT-PCR Kit (96)†	For 96 reactions: Master Mix, Positive Control, Negative Control	282305

* Available only on request.

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

Product	Contents	Cat. no.
pigtype CSFV E^{rns} Ab (5)*	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	272303
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 HEV Ab (1)	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	272501
pigtype 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
pigtype Trichinella Ab (1)	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273501
pigtype Toxoplasma Ab (1)*	For 96 reactions: 1 Test Plate (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	273401

* Other kit sizes are available; see **www.qiagen.com**.

QIAGEN offers 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 *bactotype*®, *cador*®, *cattletype*®, *flocktype*®, *pigtype* and *virotype* products.

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.

Notes

Notes

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. 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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