

# pigtype<sup>®</sup> CSFV E<sup>rns</sup> Ab Handbook

For the detection of antibodies to the E<sup>rns</sup>  
protein of the *Classical Swine Fever Virus*

Licensed in accordance with § 11 (2) of the German Animal Health Act  
MA No.: FLI-C 006

**REF** 1 plate (cat. no. PT272301)

**REF** 5 plates (cat. no. PT272303)

**REF** 20 plates (cat. no. PT272305)\*



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\* available only on request

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## Kit contents

<b>pigtype CSFV E<sup>rns</sup>Ab</b>	<b>(1)</b>	<b>(5)</b>	<b>(20)</b>
<b>Cat. no.</b>	<b>PT272301</b>	<b>PT272303</b>	<b>PT272305*</b>
<b>Number of plates</b>	<b>1</b>	<b>5</b>	<b>20</b>
Test Plate: microtiter plate with 96 wells, coated with non-infectious CSFV E <sup>rns</sup> antigen	1	5	20
Sample Diluent, ready to use	1 x 60 ml	1 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	3 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 CSFV E<sup>rns</sup> Ab is a double antigen ELISA for the detection of antibodies to the E<sup>rns</sup> protein of the *Classical Swine Fever Virus* in porcine (domestic pigs, wild boar) serum and plasma samples.

The kit is approved by the Friedrich-Loeffler-Institute and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-C 006) 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 CSFV E<sup>rns</sup> Ab is tested against predetermined specifications to ensure consistent product quality.

## Storage

The components of the pigtype CSFV E<sup>rns</sup> 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](mailto: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 CSFV E<sup>rns</sup> Ab enables rapid and reliable detection of antibodies to the E<sup>rns</sup> protein of the *Classical Swine Fever Virus* (CSFV) in porcine serum and plasma samples. CSFV belongs to the genus *Pestivirus* of the family *Flaviviridae* and causes severe and highly contagious disease in *Suidae* (e.g., domestic pig and wild boar). Therefore, Classical Swine Fever (CSF) is of worldwide importance. As CSF shows highly variable clinical symptoms, laboratory diagnostic methods are necessary for the diagnosis of an infection.

Animals infected with CSFV develop antibodies to different antigens, e.g., the viral envelope proteins E<sup>rns</sup> and E2. The pigtype CSFV E<sup>rns</sup> Ab is a highly sensitive and specific solution for detection of antibodies to the E<sup>rns</sup> protein in porcine serum and plasma samples. Therefore, pigtype CSFV E<sup>rns</sup> Ab is suitable as a CSF screening test.

Standard serological methods cannot distinguish between naturally infected and vaccinated animals. Differentiation of vaccinated animals from those naturally infected with CSFV (DIVA) is possible by immunization with marker vaccines, which do not induce a CSFV immune response specific for the E<sup>rns</sup> protein (e.g., E2 subunit vaccines or recombinant vaccines lacking CSFV E<sup>rns</sup>). In this case, pigtype CSFV E<sup>rns</sup> Ab can also be used as a reliable companion discriminatory test.

## Principle

The pigtype CSFV E<sup>rns</sup> Ab is a double-antigen ELISA. The microtiter plate is coated with recombinant CSFV E<sup>rns</sup> antigen. During sample incubation, antibodies to the CSFV E<sup>rns</sup> protein 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 to the CSFV E<sup>rns</sup> protein 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 the antibodies to the CSFV E<sup>rns</sup> protein 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.

**Note:** For reliable results and to avoid potential non-specific reactions due to residual conjugate, make sure to fill each well completely with Wash Buffer (approximately 400 µl) during washing steps. If possible, use an ELISA washer and the overflow mode setting.

# 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 **1:10** in Sample Diluent (e.g., dilute 25 µl sample in 225 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  $\mu$ l each of the Negative Control (in duplicates), Positive Control (in duplicates) and the 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. Cover the Test Plate.

1b. Pipet 100  $\mu$ l each of the Negative Control (in duplicates), Positive Control (in duplicates) into the wells of the Test Plate. Dispense 90  $\mu$ l of Sample Diluent into each sample well of the Test Plate and add 10  $\mu$ l 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 60 min at 37°C.

3. Remove solution from the wells by aspiration or tapping.

4. Rinse each well 3x with **400  $\mu$ l** of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.

**Note:** If possible, use an ELISA washer and the overflow mode setting.

5. Pipet 100  $\mu$ l ready to use Conjugate into each well and incubate for 60 min at 37°C.

6. Remove solution from wells by aspiration or tapping.

7. Rinse each well 3x with **400 µl** of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.  
**Note:** If possible, use an ELISA washer and the overflow mode setting.
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  $\geq 0.7$ .
- The mean value (MV) of the measured OD value for the Negative Control (NC) must be  $\leq 0.3$ .

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:

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

## Interpretation of the results

- **Samples with S/P-ratio < 0.3 are negative.**  
Antibodies to CSFV E<sup>ms</sup> could not be detected.
- **Samples with S/P-ratio ≥ 0.3 and < 0.5 are suspect.**  
Samples with suspect results should be retested.
- **Samples with S/P-ratio ≥ 0.5 are positive.**  
Antibodies to CSFV E<sup>ms</sup> were detected.

**Recommendation:** In order to assess suspect, weak positive or questionable positive test results, samples may be heat-treated (30 min, 56°C) before re-testing. In case of repeated suspect results that cannot be clarified by other serological methods, we recommend testing the herd for circulating virus using molecular methods, e.g., the virotype CSFV RT-PCR Kit. When using a live marker vaccine, molecular techniques that are strain specific may be appropriate for CSFV detection.

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](http://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 CSFV E<sup>ms</sup> Ab

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## Change index

Handbook	Version	Change
HB-1933-005	May 2018	INDICAL design

# Quick guide for pigtype CSFV E<sup>ns</sup> Ab

Sample dilution:

Serum, plasma 1:10; mix well

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<b>Step</b>	
1. Sample	100 µl/ well
2. Incubation	60 min at 37°C
3. Wash	3 x 400 µl*
4. Conjugate	100 µl/ well
5. Incubation	60 min at 37°C
6. Wash	3 x 400 µl*
7. TMB	100 µl/ well
8. Incubation	10 min at RT
9. Stop	100 µl/ well
10. Read	450 nm

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\* If possible, use an ELISA washer and the overflow mode setting.

## Data interpretation

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<b>Negative</b>	<b>Suspect</b>	<b>Positive</b>
S/P < 0.3	0.3 ≤ S/P < 0.5	S/P ≥ 0.5

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