PIGTYPE® CSFV Ab

ELISA Test Kit for Detection of Antibodies to Classical Swine Fever Virus

Labor Diagnostik Leipzig

Instructions for Use

In vitro Diagnostic Kit for Veterinary Use Only.

Applications

PIGTYPE® CSFV is a competitive enzyme immunoassay for the detection of antibodies to Classical Swine Fever Virus (CSFV) in swine serum and plasma samples.

General Information

Classical Swine Fever (CSF) is a viral disease which has causes serious economic losses world wide. CSFV infection occurs under natural conditions in all pigs, i.e. domestic pigs and wild boar. Surveillance testing for CSFV antibodies is a reliable and cost efficient method for monitoring and control of classical swine fever.

Description of the Test Principle

The assay is a competitive enzyme immunoassay. Microtiter plate wells are coated with CSFV antigen. For testing, ELISA plates are incubated with an equal mixture of serum and anti-CSFV antibody. During incubation, CSFV antibodies present in test sample and HRP conjugated monoclonal antibodies competitively bind to the antigens in the well. Following this incubation, all unbounded material is removed by aspiration and washing before adding a substrate solution. In case anti-CSFV antibodies are present in the sample, the conjugate is blocked from reacting with the antigen. Therefore a positive result is indicated by no or very low development of blue colour. In case of a negative result, the conjugate can bind to the antigens in the plate and react with the substrate solution causing blue colour development. The reaction is stopped by adding a stop solution and colorimetric reading will be performed by using a spectrophotometer at 450nm and 620nm. The absorbance correlates with the amount of specific antibody in the test sample.

Reagents

		5 plate kit
1.	Test plate, contains 12 micro titre strips with 8 wells, coated with CSFV antigen	5
2.	Negative Control, CSFV-negative pig serum, in buffer with protein stabilisers and preservative, ready-to-use	1.5 ml
3.	Positive Control, CSFV-reactive pig serum, in buffer with protein stabilisers and preservative, ready-to-use	1.5 ml
4.	Wash Buffer (10x), Buffer Solution with Tween and preservative	250 ml
5.	anti-CSFV-HRP, Anti-CSFV horseradish peroxidase conjugate (100x concentrated) in buffer with protein stabilisers and preservative	1.2 ml
6.	Conjugate Diluent, with protein stabilisers and preservative	40 ml
7.	TMB, Tetramethylbenzidine Substrate Solution, ready-to-use	60 ml
8.	Stop Solution, contains sulphuric acide, ready-to-use (Corrosive!)	80 ml
9.	Adhesive plate sealer	5
10.	Instructions for use	1

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Additional material and equipment required

Beakers, measuring cylinders, analytical pipettes, multichannel pipettes, disposable pipette tips, pipetting troughs, microtitre plate spectrophotometer, tubes or plates for diluting the samples, distilled water

Precautions and Warnings

Store the reagents at 2-8 °C and only bring them to room temperature (18-25 °C) immediately before use. In case of salt crystallisation in the 10x Wash Buffer dissolve the salt crystals by mixing and careful warming. Wash Buffer (10x, bottle 4) and Stop Solution (bottle 8) may be stored at room temperature (18-25°C) to avoid salt crystallisation. Store the remaining test strips in the re-sealed pack with desiccant at 2-8 °C until next use. The test strips can be stored at least for 6 weeks after opening the plate pack.

Store the TMB Substrate Solution in the dark and do not expose to intense light or to sunlight during the performance of the test. The components of the test kit may not be contaminated or mixed with components from other batches. Do not use the components of the test kit past expiration date. The water used for diluting the wash buffer concentrate, particularly water from ion-exchange plants, may interfere with the reaction if it is not pure enough. Water of the quality of double distilled water or highly purified water (Milli-Q) is recommended.

The test should only be performed by persons qualified for laboratory work. To guarantee the precision of the results, it is absolutely essential to observe the usual precautions for ELISA procedures, including the use of carefully purified glass materials, careful pipetting and rinsing during the test, and keeping to constant times during the colour reaction. Some components of the kit contain dangerous substances (Merthiolate, sulphuric acid). All sample residues and objects which have come into contact with samples must be decontaminated or disposed as potentially infective material.

Preparation of the Reagents

- Wash buffer

Mix well to ensure dissolution of any precipitated salt crystals.

Dilute the Wash Buffer (10x) 1:10 in distilled water, e.g., dilute 50 ml Wash Buffer (10x) in 450 ml distilled water for one test plate and mix well.

Wash buffer is stable at room temperature (18-25°C) for one week.

- anti-CSFV HRP Conjugate

The enzyme conjugate concentrate must be diluted 1 to 100 with Conjugate Diluent before use. Prepare the conjugate solution immediately before use and mix well.
For one plate prepare 60 µl conjugate + 5.94 ml of Conjugate Diluent.

Conjugate solution is stable at room temperature for 1 hour and at 2-8°C for 4 hours.

Preparation of the Samples

- Serum/ Plasma Samples

In this assay samples are used undiluted. Fresh, refrigerated, or previously frozen serum or plasma may be tested. Be sure to change pipette tips for each sample. Controls are ready-to-use, do not dilute them.

Any visible particulate matters in the sample should be removed by centrifugation at 3,000 rpm for at least 20 minutes.
If specimens are not immediately tested they should be refrigerated at 2-8°C. For storage periods more than three days, freeze the specimen at -20°C or below. Samples should be brought to room temperature and mixed well prior to use.
Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

Test Procedure

All reagents should equilibrate to room temperature (18-25 °C) before use.

1. Obtain the number of Test plates needed. Record the positions of the controls and samples on the Test plate in a test protocol, e.g. Negative Control = A1/A2; Positive Control = B1/B2; other positions for samples

   Always run controls in duplicates!

2. Dispense 50 µl of controls or serum or plasma samples into the appropriate wells.
   Add 50 µl of anti CSFV-HRP (1:100 dilution in conjugate diluent) to each well. Mix the contents of the microwells by gently tapping the plate or use a shaker for microtiter plates.

3. Seal the plate and incubate for 2 hour at room temperature (18-25°C) or alternatively incubate the plates sealed or in a humid chamber overnight at 2-8°C.

4. Wash 6x with 350 µl wash solution per well. Tap the plate to remove remaining liquid from the wells.

   Note: In rare cases when testing haemolytic samples, traces of blood cells may remain in the wells. For those wells the washing procedure should be repeated until all traces of blood are removed.

5. Add 100 µl TMB Substrate Solution to each well and incubate for 10 min at room temperature in the dark. Start timing when the first well is filled.

6. Stop the reaction by adding 100 µl of Stop Solution per well. Add Stop Solution in the same order as the Substrate Solution.

7. Calibrate the spectrophotometer against air as blank. Measure the optical density (OD) at 450 nm within 60 min after stopping the reaction. Measuring at 450 nm and reference wavelength (620-650) is optional.

Calculation

Abbreviations:
- MV: mean value
- NC: Negative Control (wells A1/A2)
- PC: Positive Control (wells B1/B2)
- OD: measured optical density

1. Test Validation

Valid CSFV Ab ELISA results are obtained when the average OD450 value of the Negative Control is more than 1.000 and the Positive Control value is less than 0.500. If either of these values are out of range, CSFV Ab ELISA should be considered invalid. For invalid tests, technique should be revised and the test repeated.

2. Calculate blocking percentage of each test sample and of the PC:

Calculate the mean absorbance of the Negative Controls, and then calculate the PI (Percent inhibition) value, using the formula percent inhibition

$$PI = \frac{(MV\ OD_{NC} - OD_{sample})}{MV\ OD_{NC}} \times 100$$

For example:
- Mean OD Negative Control : 1.892
- OD sample : 1.520
- PI value = 19.7 This sample is considered negative

3. Interpretation of results

Samples with PI- values	<35 are	negative.
Samples with PI- values	≥35 but <40 are	suspect
Samples with PI- values	≥40 are	positive.

Suspect samples should be retested in duplicates. In case they are suspect again, a new sample should be collected and the animal retested.