

GENLISA® Classical Swine Fever Virus Antibodies ELISA

REF : KAD1006

(previous REF No: KBVE125)

Ver 2.1

RUO

Enzyme Immunoassay for the Qualitative Screening of Classical Swine Fever Virus
Antibodies levels in porcine serum

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF KAD1006  96 tests

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GENLISA® Classical Swine Fever Virus Antibodies ELISA

Introduction:

Classical Swine Fever Virus Antibodies is a severe, acute, highly contagious infection of Pig caused by Classical Swine Fever Virus. Classical Swine Fever Virus is a major bacterial pathogen of swine, involved in meningitis, arthritis, septicemia, and acute death, among other clinical syndromes. This kit is based on antigen expression .equipped with a full range of diagnostic reagents and materials, one box can detect 96 serum sample (or partial) according to the circumstance.

Intended Use:

The GENLISA® Classical Swine Fever Virus Antibodies ELISA is used to detect Classical Swine Fever Virus antibodies in porcine serum. The kit aids in assessing the immunity conditions against Classical Swine Fever Virus in pig farms and also helps investigate epidemiology of Classical Swine Fever Virus.

Principle:

The method employs indirect ELISA technique. Classical Swine Fever Virus Antigen is pre-coated onto microwells. Samples and controls are pipetted into microwells and antibodies to Classical Swine Fever Virus present in the sample and control are bound by the capture antibody. Then, HRP Conjugate is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Classical Swine Fever Virus Antibody in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Classical Swine Fever Virus Antigen coated Microplate – 96 wells
2. HRP Conjugate - 11 ml
3. Negative Control - 750 ul
4. Positive Control - 750 ul
5. Sample Diluent - 25 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.



Handling/Storage:

1. All reagents should be stored at 2°C to 8°C for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.

GENLISA® Classical Swine Fever Virus Antibodies ELISA

3. Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. TMB Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Sample Preparation and Storage:

1. Ensure the samples which are pig serum should be collected with no bacteria.
2. Hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.
3. Samples to be diluted 1:40 using Sample Diluent provided in the kit.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate samples gently in order to ensure homogeneity.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 25 ml of (20X) Wash Buffer in 475 ml of DI water.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Classical Swine Fever Virus. High Dose Hook Effect is due to excess of antibody for very high concentrations of Classical Swine Fever Virus present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Classical Swine Fever Virus concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Classical Swine Fever Virus.
4. It is recommended that all Controls and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at 37°C.
2. Pipette **100 ul** of **Positive Control, Negative Control and Diluted Samples** into the respective wells.
3. Cover the plate and incubate for 30 minutes at 37°C.
4. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

GENLISA® Classical Swine Fever Virus Antibodies ELISA

5. Add **100 ul** of **HRP Conjugate** into each well.
6. Cover the plate and incubate for 30 minutes at 37°C.
7. Repeat the wash step as mentioned in step no. 4.
8. Add **100 ul** of **TMB Substrate** in each well.
9. Incubate the plate at 37°C for 10 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
10. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
11. Read the absorbance at 450 nm with a microplate reader.

Interpretation of Results:

The result is judged by S/P value,
 $S/P = (\text{Sample OD}_{450/630} - \text{NCx}) / (\text{PCx} - \text{NCx})$, NCx means Negative control's average OD_{450/630} value,
PCx means Positive control's average OD_{450/630} value.
If S/P ≥ 0.25, it is positive; less than 0.25, it is negative.

Validity of the test:

The test is valid if the following conditions are met,
Mean Absorbance of Negative Control < 0.40
Mean Absorbance of Positive Control ≥ 0.60

Safety Precautions:

- **This kit is For Research Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from porcine body fluids or organs used in the preparation of this kit were tested and found negative for circovirus. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.
 - Do not smoke, eat or drink while handling kit material.
 - Always use protective gloves.
 - Never pipette material by mouth.
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit



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SYMBOLS KEY

	Coated Microtiter Plate (96 wells)
	Controls
	Conjugate Horseradish Peroxidase
	Sample Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature