Operating instruction

«LTELISA ASF» indirect ELISA kit
for detection antibody to African Swine Fever Virus in swine serum

REF LT-E-ASF-01

Version: 002-2017-04

General Description:

This test kit designed for antibodies detection against African Swine Fever (ASF) in the swine and wild boar samples of sera.

Wells of polystyrene microwell strips are coated with ASF recombinant virus specific antigen and any specific antibodies from serum are bound to immobilized antigen. Such antigen-antibody complexes are further detected with horseradish peroxidase (HRP)-labeled recombinant protein G conjugate, which binds to all IgG subclasses from swine serum. Addition of enzyme substrate (chromogen TMB) results in development of blue colored product in the case HRP-labeled conjugate is present in the well. Strong color development indicates the presence of IgG to ASF in the sample sera. Very weak or no color development indicates the absence of IgG to ASF in the sample sera.
This kit allows assaying of 92 samples, without control samples.

Kit Contents:

Component	Quantity
Antigen-coated plate	1 plate
Conjugate (x100)	1 vial, 0.1 ml
Wash solution concentrate(x30)	1 vial, 15.0 ml
Stop reagent	1 vial, 6.0 ml
Serum dilution buffer	1 vial, 12.0 ml
Substrate Solution (TMB)	1 vial, 11.0 ml
Positive control (C+)	1 vial, 0.1 ml
Negative control (C–)	1 vial, 0.1 ml
Sticky film	1 piece
Operating instruction	1 piece

Preparation of reagents:

1. Warm up reagents at room temperature (18–22) ºC during 30 minutes.

2. Prepare (1X) Wash solution. Intensively shake Wash solution concentrate (x30) (15ml) and dilute with 435.0 ml of distilled or deionized water. If Wash solution concentrate contains sediment dissolve crystals by heating at (35–37) ºC. Keep (1X) Wash solution at temperature (2–8) ºC no longer than 5 days in clean vial tightly closed.

3. Prepare conjugate solution. Conjugate solution (1X) has to be prepared before use. Dilute one part of Conjugate (x100) with 99 parts of Wash solution (1X) in a clean vial. Mix well avoiding foaming.

For example:

For one strip add 1.0 ml of Wash solution (1X) to clean bottle, and then add 0.01 ml of Conjugate (x100). Mix well by pipetting.

For plate add 10.0 ml of Wash solution (1X) to clean bottle, and then add 0.1 ml of Conjugate (x100). Mix well by pipetting.

4. Prepare serum specimens. If samples containing aggregates and/or precipitate should be clarified by centrifugation for 10 minutes at 2500-3000 RPM. Serum samples may be stored at (2-6) °C no longer than three days. It is allowed to store the serum samples at - 20°C (or lower) for three months and at - 70 °C for two years. Do not freeze and thaw sera specimens twice. Sera with hemolysis, hyperlipidemia or bacterial contamination are not suitable for the analysis!

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Test Procedure:

1. Remove protective foil from antigen-coated plate and add 0.09 ml Serum dilution buffer into all wells of the plate.

2. Add 0.01 ml of serum samples into wells of the plate leaving free of the first 4 wells (A1, B1, C1, D1) for Positive and Negative controls.

3. Add 0.01 ml of the Positive control (C+) into wells A1, B1, and 0.01 ml of the Negative control

(C–) into wells C1, D1. Carefully pipette the mixture in wells. Take care not to spill samples from well to well. During the pipetting solution in wells may be changed in color. Stick the plate by enclosed film and incubate at temperature 37.0ºC for one hour.

4. Rinse the plates 4 times with 0.35 ml of 1X Wash solution using microplate washing machine: aspirate the well contents completely, fill up the wells at each rinse and aspirate completely. Make sure that no fluid remains on the strips and strip holder after the last aspiration. If necessary, dry the plate by tapping on absorbent paper.

5. Add 0.1 ml of Conjugate solution (see above) into each well. Stick a plate by enclosed film and incubate at temperature 37.0ºC for 30 minutes.

6. Rinse the plates 6 times with **0.35 ml** of 1X Wash solution following washing procedure described in point #4.

7. Add 0.1 ml of Substrate Solution (TMB) into each well. Incubate plate at 20–250C for 30 minutes. Avoid leaving the plate in direct sunlight.

8. Stop color reaction by adding of 0,05 ml Stop Solution to each well.

9. Measure the optical density (OD) of the controls and samples at 450/620 nm using a microplate photometer. To prevent the fluctuation in OD values measure OD within 3 minutes after reaction was stopped.

Result interpretation:

1. Calculate the mean OD-values for each of the controls (mean OD Negative Control and maen OD Positive Control);
2. Calculate the percent positivity (PP) values for negative control as well as samples, using the following formula:

$$PP = \frac{Mean\ OD\ sample\ or\ Negative\ control}{Mean\ OD\ Positive\ control} \times 100$$

For example:

$$PP = \frac{0{,}599}{2{,}690} \times 100 = 22.26\%$$

3. Analysis is considered correct, if mean value of OD Positive Control are greater than 1.5, mean value of PP Negative Control are less than 8.
4. Results of the analysis for investigated samples are considered **Positive** if PP samples are equal or greater than 9%. Results of the analysis for investigated samples are considered **Negative** if PP samples are less than 9%.
5. 9% - the constant value is established by the manufacturer and may be changed in various LOT numbers.

Storage and Stability:

Store all reagents at 2-8ºC. Do not freeze. Reagents will remain stable until the expiration date when stored as recommended. Do not use test kit past the expiration date printed on the label.

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