Porcine Classical Swine Fever Virus Antigen（CSFV Ag) ELISA Kit

to detect Classical swine fever virus antigen (CSFV-Ag) in Porcine serum, blood plasma, and other related tissue liquids

INSTRUCTION MANUAL

FOR ELISA KIT No: LT52003AYSL

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INTENDED USE

This kit is used to detect the classical swine fever virus antigen in the sample of porcine serum, blood plasma, tissue liquid samples or related biological solutions. For *In vitro* use only.

MANUAL VERSION 1.01C

INTRODUCTION

ASSAY PRINCIPLE

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to detect Porcine Classical swine fever virus antigen (CSFV Ag) in samples. Sample containing Porcine Classical swine fever virus antigen (CSFV Ag) is added to a precoated micro-elisa well. Next an HRP-conjugate reagent is added to the wells. After incubation and washing, the unbound enzyme is removed and Chromogen Solution A and B are added. The color of the liquid changes to blue, and in the effect of an acid the color finally become yellow. The intensity of the color is measured at 450 nm. In order to infer the presence of CSFV Ag in the sample, the CSFV Ag ELISA Kit includes a set of controls. The controls are assayed at the same time as the samples and allow the operator to produce a cutoff value. The existence or not of CSFV Ag in the samples is then determined by comparing the O.D. of the samples to the CUT OFF.

MATERIALS SUPPLIED WITH THIS KIT

Name	Content
Microelisa stripplate	12*8strips
Negative control	0.5ml
Positive control	0.5ml
HRP-Conjugate reagent	10.0ml
20X Wash solution	25ml
Sample Diluent	6.0ml
Chromogen Solution A	6.0ml
Chromogen Solution B	6.0ml
Stop Solution	6.0ml
Closure plate membrane	2
User manual	1

Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips

4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

ASSAY PROTOCOL

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000×g. Remove serum and assay immediately or aliquot and store samples at -20℃ or -80℃.Avoid repeated freeze-thaw cycles

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 30

minutes at 3000×g at 2-8℃ within 30 minutes of collection. Store samples at -20℃or -80℃. Avoid repeated freeze-thaw cycles.

Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃or -80℃. Avoid repeated freeze-thaw cycles

Note: Samples should be centrifuged adequately and no hemolysis or granule should be allowed.

REAGENT PREPARATION

Bring reagent to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

Wash buffer - 20×wash solution: Dilute with Distilled or deionized water 1:20.

ASSAY PROCEDURE

1.Bring all reagents to room temperature before starting assay procedure. It is recommended that all samples are performed in duplicate.

2. Separately add 50µl Positive control and 50µl Negative control to the Positive and Negatively marked wells.

3. Add the unknown test sample 10µl followed by 40µl sample diluent to sample testing well.

3. Add 100µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.

4. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with wash solution (300-400µl) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each wash is essential to good performance. After the last wash, remove any remaining wash solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 50µl chromogen solution A and 50µl chromogen solution B to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.

6. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

CALCULATIONS

1. Test validity:

The average value of Positive control wells≥0.8;

The average value of Negative control wells ≤0.20.

2. Calculate Critical (CUT OFF):

CUT OFF = the average of Negative control well + 0.15.

3. Inference:

Negative Result: Sample OD< Calculate Critical (CUT OFF) is Negative.

Positive Result: Sample OD≥ Calculate Critical (CUT OFF) is Positive.

PRECAUTIONS

1.Do not substitute reagents from one kit to another. Conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer.

2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.

3. Shake all reagents before using. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C) before using.

VALIDITY & STORAGE: twelve months .

Storage： 2-8°C.