VERSION 1.01

CLASSICAL SWINE FEVER VIRUS ANTIBODY ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Classical Swine Fever Virus Antibody ELISA Test Kit

Catalogue Number. IP100173

Product Usage

The Classical Swine Fever Virus (CSFV) antibody ELISA Test kit is used for detection of Classical Swine Fever Virus antibody in porcine serum; assessment of immunity conditions against Classical Swine Fever Virus in the pig farm and investigation of the epidemiology of the Classical Swine Fever Virus.

Principle

The Classical Swine Fever Virus(CSFV) antibody ELISA test kit is made from the antigen coated microtiter plate(coated with CSFV antigen) and other reagents. It applies the Solid-phase ELISA principle to CSFV-Ab in serum, then add enzyme conjugate to specifically bind with complex of coated antigen+CSFV-Ab+enzyme labeled anti-pig-IgG antibody on the microplate. With the TMB substrate, it will generate an amount of color. The depth of color is relative with the content of the CSFV-Ab, when the value of color is greater than the cut-off value, the pigs are vaccinated well or natural infected exist.

Technical specifications

96 wells × 2.

Components

1	CSFV- Antigen coated microplate	96T X 2	
2	CSFV- Negative control serum	1.5 mL/tube	green lid
3	CSFV-IgG Positive control serum	1.5 mL/tube	red lid
3	Enzyme conjugate	22ML	yellow lid
4	20×concentrated washing solution	50ml	white lid
5	Substrate A	1 bottle	orange lid
6	Stop solution	12ml	blue lid
7	Sample diluent solution	50 ml	transparent lid
8	Adhesive Foil	2 pieces	
9	Instruction	1 pieces	

Material required not provided

1 Microplate Reader (Dual-wave length: 450/630 nm).
2 Microplate Washers.
3 Micropipettes, adjustable (Single-wave length 1-100ul、0.5-10ul、multi-wave length 30-300ul).
4 Constant temperature box o r water bath box.
5 Oscillators.

6 Disposable tips (10ul, 200ul)
7 Deionized water

Sample requirement

1 The samples are porcine serum, which should be collected with no bacteria. The storage time should be less than 1 week at 2-8 ℃, if for long term, it should be kept at -20℃.
2 Avoid to use the samples with severe hemolysis, precipitate, contaminated by bacteria or protein suspension.
3 The EDTA, heparin sodium and other anticoagulants will not affect the results.

Preparation

1.Bring ELISA reagents to the room temperature (20-25 ℃) for 30 min to get best results. Microplate should return to room temperature and dry before open package.
2. Sample dilute: Dilute sample with the sample diluent at 40 times.(5ul serum + 195ul sample diluent), the diluted sample need to mix evenly to get better results.
3. Washing solution preparation: Dilute the 20×concentrated washing buffer with deionized water at 20 times. (50ml 20×concentrated washing buffer + 950ml deionized water) It is normal if there is crystallization in the 20×concentrated washing buffer, put at 37℃ until completely dissolved.

Operation procedures

1.Take pre-coated microtiter strips (Can unseal for several time use as per sample quantity), add 100μL diluted serum to test well, meanwhile set 2 wells for Negative control, 2 wells for Positive control separately. Add 100 μL Negative/Positive control to its well. Shake softly, cover and incubate at 37℃ for 30 min.
2.Pour the liquid out of the wells, add diluted washing solution to each well fully, be static for 10s, pour out. Wash3 times, at last pat to dry on absorbent paper.
3. Add 100 μL Enzyme Conjugate to each well, and incubate at 37℃ for 30 min.
4. Repeat the step 2(washing). Remember pat to dry on absorbent paper at last.
5. Add 100 μL substrate to each well, mix properly, react for 10 min at 37℃ at dark.
6. Add 50 μL stop solution in each well, and measure the result within 10 min(shake evenly before read result, no spill).
7. Read OD value with ELISA reader at 450nm/630nm.

Results

For the assay to be valid, the positive control wells' average OD value must be greater than or equal to 0.6, and the negative control wells' average OD value is less than 0.1. Otherwise the test is invalid, need test again.
The result is judged by S/P value,
S/P=(Sample OD450/630- NCx(—))/(PCx(—)- NCx(—)), NCx(—) means Negative control's average OD450/630 value, PCx(—) means Positive control's average OD450/630 value
If S/P≥0.2, it is positive; less than 0.2, it is negative.

Precautions

1. Wear glove and working clothes when operate, treat the test kit as containing infectious material.
2. Experiment rubbish should be dealt with high pressure steam sterilization at 121 ℃ for 30 minutes, or treated with 5.0g/L sodium hypochlorite disinfectant for 30 minutes, then discard.

3. Micro Well plate removed from the refrigerated environment should be balanced moisture to dry at room temperature, and then can be opened. Put back unused MicroWell plate into dry foil bag and sealed at 4 ℃. Unused liquid reagent should cover caps, store at 2-8 ℃ in dark with other group components.
4. If the 20×concentrated washing buffer appears crystal, it is normal, put at 37℃ until been dissolved.
5. Should use Micropipette to add sample and reagents, and often proof its accuracy.
6. When adding washing buffer, should be full but no overflow, avoid appearing free enzyme at mouth of well or cross pollution between wells.

Storage: store at 2-8℃, dark, sealed, dry place, no frozen.
Expiry date: 12 months; date of production is on box.