VERSION 1.01

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) ANTIBODY ELISA KIT

MANUAL

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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Antibody ELISA Kit

Catalogue Number. IP100177

Brief

The Porcine reprodutive and respiratory syndrome virus antibody test kit (PRRSV Ab) is used for the detection of PRRSV antibodies in swine serum; assessment of immunity conditions against porcine reprodutive and respiratory syndrome virus in the pig farms; and investigation epidemiology of the PRRSV.

Principle

The PRRSV Ab ELISA test kit is made from the antigen coated microtiter plate, goat-anti-pig IgG-HRP and other reagents. It applies the indirect ELISA principle to test the antibodies against PRRSV in porcine serum. In the test, the coated antigen combine with PRRSV-IgG in serum, then add IgG-HRP to specifically bind with complex of antibody-antigens 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 PRRSV-IgG.

Technical specifications

96 wells × 2.

Components

1	PRRSV Antigen coated microplate	96T X 2	
2	PRRSV IgG Negative control serum	1.5 mL/tube	Green lid
3	PRRSV -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	1 bottle	Orange lid
6	Stop solution	12ml	Blue lid
7	Sample diluent solution	50 ml	Transparent lid
8	Adhesive Foil	6 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.

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 .Adding sample: Take out the required coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. For single-wave length test, set one blank control well, add nothing; for double-wave length test, do not set blank control well. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100 µL/well. Others are wells for samples, add 100µL/well of Sample diluent solution, then add 1 µL serum sample separately (there will be color change after adding sample).
2. Mix gently for 30s, incubate at 37℃ for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution(dillute the Wash Concentrate at 20 times with deionized water) into each well fully, stand for 1 min. Repeat 5 times, at last time pat to dry on absorbent paper.
4. Add 100 µL enzyme comjugate into each well (except the blank well, also do not add any liquid to blank well).
5. Cover plate with new adhesive foil. Incubate at 37 ℃ for 30 min.
6. Repeat step 3(washing).
7. Add 50µL substrate A and substrate 50µL B into each well, mix properly, incubate for 15 min at 37 ℃ in the dark with new adhesive foil.
8. Add 50µL stop solution into each well, mix gently and determine the result within 5-30 min.
9. For single-wavelength test, measure the A value with a photometer at 450 nm, set zero for the blank well, and read A value of each well; For double-wavelength test, measure the A value with a photometer at 450 nm (Reference-wavelength: 630 nm), read A value of each well.

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.

Interpretation of the result

1. Severe hemolysis, fiber protein in the serum separation is not sufficient, containing erythrocytes, a precipitate, a sample with bacteria may lead to false positive.
2. Negative results may occur on individual pigs after vaccines due to individual differences or immune duration.
3. Positive results for serological diagnosis and epidemiological investigation of swine to be combined with other methods and clinical data.

Limit of test method

PRRSV-IgG can be used as the evaluation of PRRSV vaccine effect and PRRSV virus infection serological diagnostic indicators, but can not distinguish the two, if want to distinguish antibody caused by the vaccine or wild virus infection, it shout be combined with clinical data, immunization programs etc.

Product performance

1. Specificity: use this kit to detect reference serum, the compliance rate reach 100%.
2. Sensitivity: can reach max 1:5120.
3. Precision: CV（%）no bigger than 8%.
4. Stability: Store at 2℃～8℃ for 12 months or store at 37℃ for 3 days, the result can reach the above 3 standards.

Precautions

This test kit is suitable for in vitro diagnostics.
2. Wear glove and working clothes when operate, treat the test kit as containing infectious material.
3. 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.
4. 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.
5. If the 20×concentrated washing buffer appears crystal, it is normal, put at 37℃ until been dissolved.
6. Should use Micropipette to add sample and reagents, and often proof its accuracy.
7. When adding washing buffer, should be full but no overflow, avoid appearing free enzyme at mouth of well or cross pollution between wells.
8. Stop solution is corrosive, use large amount of water to wash immediately when touch the skin or clothes.

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