Porcine Reproductive and Respiratory Syndrome Virus Antibody Test Kit (PRRS X3 Ab)

CAT: AE0001

Name and Intended

The PRRS X3 test kit is an enzyme immunoassay for the detection of antibody to porcine reproductive and respiratory syndrome (PRRS) virus in swine serum or plasma using PRRSV antigens.

General Information

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is a single stranded positive RNA arteritis virus, divided into European (Type I or A) and American (Type II or B) strains, with different antigenicity. The virus causes reproductive problems (miscarriage, weak birth, stillbirth, sow death), respiratory diseases (pneumonia, difficulty breathing, delayed movement, death), and mild neurological symptoms. Causing serious economic losses is considered one of the most important diseases affecting the pig farming industry. This reagent kit can conveniently detect antibody levels in serum or plasma, and evaluate whether pigs are infected with PRRSV (natural infection or artificial immune vaccine infection).

Descriptions and Principles

The PRRS X3 antibody detection kit is an enzyme-linked immunosorbent assay designed to detect PRRSV antibodies in pig serum or plasma. The recombinant PRRSV antigens is coated on an enzyme-linked immunosorbent assay (ELISA) plate. When the tested sample is incubated in the coating well, the specific antibodies against PRRSV in the sample form an antigen antibody complex with the coated antigen. After washing away the unbound components in the coating well, horseradish peroxidase (HRP) labeled anti porcine antibodies are added, which bind to the complex in the well. Then wash off the unbound enzyme-linked antibodies and add the chromogenic substrate TMB. The depth of color reaction is positively correlated with the amount of anti PRRSV specific antibodies present in the sample.

This reagent kit is only for in vitro testing purposes.

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Reagents

Content	Volume	Quantity
PRRS Antigen Coated Plate（CP）	96 wells/plate	5
PRRS Ab Positive Control（PC）	4 mL/bottle	1
PRRS Ab Negative Control（NC）	4 mL/ bottle	1
Conjugate：HRP（HRP）	60 mL/ bottle	1
Sample Diluent（SD）	120 mL/ bottle	1
TMB Substrate（TMB）	60 mL/ bottle	1
Stop Solution (NaF)（SS）	60 mL/ bottle	1
Wash Concentrate 10×（WB）	125 mL/ bottle	1
Sealer Film（SF）	6 pieces/bag	3

Storage

Store the reagents at 2~8°C. Reagents are stable until expiration date , provided they have been stored properly .

Materials Required but Not Provided

- Precision micropipettes or multi - dispensing micropipettes
- Disposable pipette tips
- Graduated cylinder for wash solution
- 96- well microplate reader (equipped with 650 nm filter)
- Microplate washer (manual , semi - automatic or automatic system)
- Use only distilled or deionized water for preparation of the reagents used in the test
- Vortex or equivalent
- Tubes or plate for diluting samples

Precautions and Warnings

- Proper disposal of all biological materials, which may be potential sources of infection.
- TMB substrate may be irritating to the skin and eyes, avoid contact with the skin and eyes.
- Do not expose TMB to strong light or any oxidizing agent. TMB substrates should be accessed in clean glass or plastic containers.
- All reagents should be stored at 2~8°C. Equilibrate to room temperature (18~25°C) before use, and store back at 2~8°C after use.
- All wastes should be properly decontaminated prior to disposed.
- Pay attention to prevent contamination of the reagents in the kit.
- Do not use expired reagents, and do not mix kit components from different batches.
- Strict adherence to the operating instructions can obtain the best results. All processes such as pipetting, timing, and washing must be maintain precision and accuracy.
- Set up a positive and negative control for each test.
- During the test, use only deionized water or distilled water to dilute and prepare the reagents.
- The unused microorifice slats should be sealed in aluminum foil bags and stored at 2~8 °C.
- For veterinary diagnostic use only.

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Preparation of reagents

Preparation of samples

Dilute test samples 40- fold (1/40) with Sample Diluent (e.g., by diluting 5 µL of sample with 195 µL of Sample Diluent).

NOTE : DO NOT DILUTE CONTROLS.

Be sure to change tips for each sample . Samples must be thoroughly mixed prior to dispensing into the coated plate .

Preparation of Wash Solution

The Wash Concentrate must be brought to 18~25°C and mixed to ensure dissolution of any precipitated salts . The Wash Concentrate must be diluted 10- fold (1/10) with distilled / deionized water before use (e.g., 25 mL of concentrate plus 225 mL of water per plate to be assayed).

Test Procedure

All reagents must be allowed to come to 18~25°C before use . Mix reagents by gentle inverting or swirling .

1. Obtain sufficient coated plates for samples to be tested and record the sample position.
2. Add 100 µL of undiluted negative control (NC) to the coated wells, adding two wells per assay.
3. Add 100 µL of undiluted positive control (PC) to the coated wells and add two wells per assay.
4. Add 100 µL of the diluted sample to the appropriate wells.
5. Seal the sealing film (SF) and incubate at 18~25°C for 30 minutes (± 2 minutes).
6. Pipette the liquid from each hole and discard it into the waste container.
7. Wash the plate wells with 300 µL of wash solution (WB 1×) for a total of 4 washes. The liquid in the wells should be sucked off after each wash. Note: Drying of coated wells should be avoided. After the last wash absorbent, tap the remaining wash solution buckles from each plate onto the absorbent material.
8. Add 100 µL of horseradish peroxidase-labeled anti-porcine antibody (HRP) to each well.
9. Seal the sealing film (SF) and incubate at 18~25°C for 30 minutes (± 2 minutes).
10. Repeat steps 6 and 7.
11. Add 100 µL of TMB chromogenic substrate to each well.
12. Incubate at 18~25°C for 15 min (± 1 min).
13. Add 100 µL of stop solution (SS) to each well to stop the reaction.
14. The absorbance value A (650) of the sample and control was measured and recorded.
15. Calculation results.

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How to calculate the S/P

Calculation of negative control mean ($\overline{NC}$):

$$\overline{NC} = \frac{NC1\ A_{650} + NC2\ A_{650}}{2}$$

eg:

$$\overline{NC} = \frac{0.086 + 0.090}{2} = 0.088$$

Calculation of positive control mean ($\overline{PC}$):

$$\overline{PC} = \frac{PC1\ A_{650} + PC2\ A_{650}}{2}$$

eg:

$$\overline{PC} = \frac{0.838 + 0.898}{2} = 0.868$$

Calculation of sample (S/P) values:

$$S/P = \frac{Sample\ A_{650} - \overline{NC}}{\overline{PC} - \overline{NC}}$$

eg: Sample A (650) = 1.398

$$S/P = \frac{1.398 - 0.088}{0.868 - 0.088} = 1.68$$

Determine the validity of the experiment

At the same time, conditions ① and ② are met for the experiment to be effective:

①The mean value of the negative control ($\overline{NC}$) must be less than or equal to 0.150;

②The mean value of the positive control ($\overline{PC}$) minus the mean value of the negative control ($\overline{NC}$) must be greater than or equal to 0.150.

If the experiment is invalid and the operation in the experiment is questionable, the experiment should be redone according to the operating instructions.

Judgment of the result

The positive or negative PRRS antibody is determined by calculating the ratio of the sample to the positive control S/P (Sample/Positive)。

1. If the S/P value is less than 0.40, the sample should be judged negative for PRRS antibodies.

2. If the S/P value is greater than or equal to 0.40, the sample should be judged to be positive for PRRS antibody.

攸克生命科学技术（杭州）有限公司提供免费的数据计算平均值、S/P 比值以及提供综合信息。

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