PRRS antibody ELISA Kit

PRRS Ab Test

Product Number: E30021

Product Unit: 1 plate, 96T / 2 plates, 192T / 5plates, 480T

Manufacturer Information

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1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a virus that causes a disease of pigs, called porcine reproductive and respiratory syndrome (PRRS), also known as blue-ear pig disease. This economically important, panzootic disease causes reproductive failure in breeding stock and respiratory tract illness in young pigs. The disease costs the United States swine industry around $644 million annually, and recent estimates in Europe found that it costs almost 1.5 billion euro every year.

This kit utilizes recombinant nucleocapsid protein of PRRSV(protein N), based on which indirect ELISA or lateral flow immunoassay were further developed for laboratory testing purpose.

2. Description of Test

The current PRRS Ab ELISA kit is designed to detect PRRSV antibody in swine serum samples. The 96well microtiter plate was precoated with recombinant nucleocapsid protein (protein N). During testing, samples are added into the microplate wells, in which the precoated antigen will capture the PRRSV antibody in sample and formed antigen-antibody complex. None specific antibody are discarded by a washing step. Then goat-anti-swine IgG conjugated with horseradish peroxidase (HRP) is added into each well, and further forms antibody-antigen-antibody complexes. After another washing step to remove unreacted conjugate, substrate is added and a blue color will be developed if PRRSV antibody is present. The enzyme reaction is stopped and the OD450nm value is measured. The measured intensity is positively proportional to the amount of antibody present in the sample.

This ELISA kit can be used to detect PRRS antibody in **swine serum**.

3. Precautions

- Store the kit at 2-8℃,Check the lot number and expiration date before use.
- Bring the test kit to room temperature before use. For example, take it out from the cold storage and put at room temperature for at least 30min.
- The stop solution in the kit is acidic, please make sure do not touch it with your hand or skin.
- The component of the kit is noninfectious, but the field sample shall be treated as potentially infectious. Please handle all these materials properly according to your lab regulations.
- After experiment, all lab materials shall be handled properly according to local regulations.

4. Limitations of Test

This ELISA kit is currently designed for research purpose. We recommend validating in your own lab with different methodologies to confirm the performance. If it is not used for the mentioned purpose, please contact us for help.

5. Reagent Provided

The kit contains the following items.

Item No.	Description	Quantity

1	Microplate pre-coated with PRRS antigen	5 plates
2	Positive Control	1 X 2 ml
3	Negative Control	1 X 2 ml
4	Sample buffer	2 X 60 ml
5	HRP enzyme conjugate	1 X 60 ml
6	TMB substrate	1 X 60 ml
7	Stop solution -- Not provided	1 X 60 ml
8	25X Wash buffer	1 X 60 ml
9	Kit instruction	1set

Note: Stop solution is 2M sulfuric acid, which is not provided due to air cargo shipment, which shall be prepared by client.

6. Instrument Required

- ELISA reader
- Micropipette 20-200ul
- Micropipette Multi-Channel 50-300ul

7. Reagent Preparation

- **Wash buffer**: dilute the 25xWash buffer provided in the kit with deionized water in the volume ratio of 1:24. For example, 1ml 25xWash buffer + 24ml deionized water
  The diluted wash buffer can be stored at room temperature for 2 weeks.
  No other reagent is required. Please remember to return all kit component to room temperature before use.

8. Sample Preparation

- **Positive Control / Negative Control:** dilute with sample buffer in the volume ratio of 1:19. For example: 11ul positive/negative control + 209ul sample buffer.
- **Sample:** dilute with sample buffer in the volume ratio of 1:39. For example: 10ul sample + 390ul sample buffer.
  Please freshly prepare before assay.

9. Assay Procedure

1) Make sure the kit and all test samples are returned to room temperature before use. Shake each reagent gently before adding into the well.
2) Open the kit, read the kit instruction carefully to make sure all technical points are understood clearly.
3) Take the microplate from the zip-bag, and take needed microwells, store the rest into the zip-bag. Make marks of the plate layout. **Running the test in duplicated wells** is recommended to minimize operational error.
4) Add Positive control: add 100ul positive control into the wells.
5) Add Negative control: add 100ul negative control into the wells.
6) Add Sample: add 100ul sample into the other wells.
7) Incubation: cover the plate with plate cover and incubate at **room temperature (22-27 °C) for 30min**.
8) Washing: pour the liquid out from the wells and wash with wash buffer (300ul per well) for 3 times. Tap the residue liquid against absorbent paper to make sure the plate is dry after

washing.

9) Add enzyme conjugate: add 100ul of HRP conjugate into each well. Cover the plate again and then incubate at **room temperature again for 30min**.

10) Washing: repeat the washing step again.

11) Add substrate: add TMB substrate into each well, 100ul per well. Cover the plate again and then incubate at **room temperature again for 15min**. Color reaction will occur in the plate.

12) Stop the reaction: add 50ul stop solution into each well, the color will turn yellow from blue.

13) Read the plate: using ELISA reader to read the plate at 450nm.

10. Result Determination

The test results are valid only if the Average OD value of negative control is below 0.200 and the Average OD of positive control is greater than 0.500. Otherwise, please run the analysis again with new kit.

1) Calculation of S/P:

$$\frac{\textbf{Mean OD of Sample – Mean OD of Negative Control}}{\textbf{Mean OD of Positive Control – Mean OD of Negative Control}} = \text{S/P}$$

2) Criteria of Positive and Negative results.

Positive: **S/P ≥ 0.4** **Negative**: **S/P < 0.4**

11. Storage and expiration

The kit shall be store at 2-8°C, avoid direct sunlight.

The valid period is 12 months.

12. References

(1) OIE manual, http://www.oie.int/doc/ged/D13986.PDF

(2) Seuberlich T, Tratschin JD, Thür B *et al*, Clin Diagn Lab Immunol. 2002,9(6):1183-91, Nucleocapsid protein-based enzyme-linked immunosorbent assay for detection and differentiation of antibodies against European and North American porcine reproductive and respiratory syndrome virus.