PRRS antibody ELISA Kit

PRRS Ab Test

Product Number: E30021

Product Unit: 1plate, 96T ; 2 plate, 192T ;5 plate, 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 or plasma 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 antibody 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 or plasma**.

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	Description	Quantity	Quantity	Quantity
1	Microplate pre-coated with PRRS antigen	1 plate	2 plates	5 plates
2	Positive Control	1 X 1 ml	1 X 1 ml	2 X 1 ml
3	Negative Control	1 X 1 ml	1 X 1 ml	2 X 1 ml
4	Sample buffer	1 X 25 ml	2 X 25 ml	4 X 25 ml
5	HRP enzyme conjugate	1 X 25 ml	1 X 25 ml	3 X 25 ml
6	TMB substrate	1 X 25 ml	1 X 25 ml	3 X 25 ml
7	Stop solution	1 X 20 ml	1 X 20 ml	2 X 20 ml
8	25X Wash buffer	1 X 30 ml	1 X 30 ml	3 X 30 ml
9	Kit instruction	1set	1set	1set

6. Instrument Required

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

7. Reagent Preparation

- Make sure the kit and all test samples are returned to room temperature (20-25°C) before use. Shake each reagent gently before adding into the well.
- The positive control, negative control, enzyme-labeled antibody, serum diluent, substrate and stop solution can be used directly.
- Wash buffer (25X): dilute the 25X wash buffer provided in the kit with deionized water in the volume ratio of 1:24. For example, 10ml 25X wash buffer + 240ml deionized water. The diluted wash buffer can be stored at 2-8°C for 3 days.

8. Assay Procedure

1. Take the microplate from the zip-bag, and mark the location of the sample.
2. Add 100ul negative control into two wells (100ul/well).
3. Add 100ul positive control into another two wells (100ul/well).
4. Add 100ul serum sample diluent (dilute the serum sample with sample buffer in the rate of 1:49, for example, 2ul serum sample + 98ul sample buffer) into other wells (100ul/well). The sucker needs to be replaced when different samples are drawn.
5. Cover the plate with plate cover and incubate at 25 °C for 30min.
6. Pour the liquid out from the wells and wash with wash buffer (300ul per well) for 5 times. Tap the residue liquid against absorbent paper to make sure the plate is dry after washing.
7. Add 100ul of HRP enzyme conjugate into each well. Cover the plate again and then incubate at 25 °C again for 30min.
8. Repeat the washing step again.
9. Add the substrate into each well, 100ul per well. Cover the plate again and then incubate at 25 °C again for 15min. note: avoid direct sunlight.
10. Add 50ul stop solution into each well to stop the reaction.

11. Using ELISA reader to read the plate at 450nm.

9. Result Determination

If Average OD value of negative control < 0.4 and the Average OD of positive control I ≥ 0.6, the test is valid. Otherwise, please run the analysis again with new kit.

1) Calculation of S/P:

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

2) Criteria of Positive and Negative results.

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

10. Storage and expiration

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

The valid period is 12 months.

11. 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.