Science for Society

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) Antibody ELISA Test Kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT01003AYSL

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MANUAL VERSION 1.02

PRINCIPLE

Porcine Reproductive and Respiratory Syndrome (PRRS) virus antibody ELISA kit is used to detect PRRS antibody in pig serum, and to evaluate the immune status of pig farm PRRS vaccine and serologically assist diagnosis of infected pigs. This kit is very sensitive to detect antibodies against PRRSV. This kit uses indirect ELISA method, purified PRRS antigen is pre-coated on micro-well strips. When testing, diluted serum sample are added, and after incubation, if there is PRRS virus specific antibody, it will combine with the pre-coated antigen, the uncombined antibody and other components are discarded with washing; and then enzyme labeled antibody is added to bind to the complex; the uncombined enzyme conjugate is removed with washing; and TMB substrate is added in micro-wells, the blue signal is developed and Enzyme catalysis is proportional to anti PRRSV antibody content in sample. Use ELISA reader at 450nm wavelength to measure the absorbance value in reaction wells after adding stop solution.

MATERIALS SUPPLIED WITH THIS KIT

1	Ag coated microplate	96T × 2	7	Substrate	22 ml
2	Negative control	1.6 ml	8	Stop solution	11 ml
3	Positive control	1.6 ml	9	Serum dilution plate	2 pieces
4	Sample diluent	100 ml	10	Adhesive Plate sealer	4 pieces
5	Washing solution 10X concentrated	100 ml	11	Instruction	1 piece
6	Enzyme conjugate	22 ml			

MATERIALS REQUIRED BUT NOT PROVIDED

1) Microplate Reader (double-wave length: 450/630 nm).

2) Precise micropipette (single-channel 1-100µl, 0.5-10µl, multi-channel 30-300µl). 3) Constant temperature box or water bath.

4) Oscillator. 5) Microplate Washer.

6) Disposable tips (10µl, 200µl). 7) Deionized water.

4. SAMPLE PREPARATION

Take animal whole blood, get serum by using regular method, the serum should bright and no hemolysis. Dilute sample at 1:50 (2ul serum to 98ul diluent) using sample diluent.

5. WASHING BUFFER PREPARATION

Return 10X Concentrated washing buffer to room temperature before use, if there are salt crystals, shake to make them dissolved, then dilute it at 10 times with distilled water or deionized water. The diluted washing buffer can be stored at 4℃ for about 1 week.

6. NOTES

1) Return all reagents to room temperature before use, shake it evenly before use, and store back to 2-8℃ after usage.

2) Do not mix use reagents from different kits and different lot no., prevent the reagents from being polluted when using.

3) Substrate and stop solution may have irritation to skin and eyes, be careful in handling them.

4) Do not expose Substrate to strong light and avoid contact with oxidant.

5) Pre-coated plates should be sealed and moisture-proof. Put back unused microwell plate into dry foil bag and seal at 2-8 ℃.

6) All wastes should be treated well to avoid pollution before discarding.

7) Strict compliance with the operating instructions can get the best results. Pipetting operation, timing, and washing of the whole process must be precise.

8) Pre-coated plates are to be disposed, do not repeat use.

7. TEST PROCEDURE

1) Take the antigen coated plate (the plate can be opened and used several times according to sample quantity each time), add the diluted serum (at 1:50 dilution) to reaction wells, 100µl/well; meanwhile, set 2 wells for positive control and 1 well for negative control, both positive control and negative control do not need to be diluted, take 100µl directly and add into these well, mix gently (do not overflow);

2) Cover it with Adhesive plate sealer; incubate at 37 ℃ for 30 minutes;

3) Open the adhesive plate sealer; discard the liquid of the well

Add diluted washing buffer to each well, 250µl/well, soak for 1min between washes, then discard the liquid, repeat the above step for 4-6 times, at last wash, flap to dry with absorbent paper;

4) Adding Enzyme Conjugate, 100µl/well, Cover it with Adhesive plate sealer, incubate at 37 ℃ for 30 minutes;

5) Open the adhesive plate sealer, discard the liquid of the well, washing 4-6 times as step 3, remember at last wash, flap to dry with the absorbent paper;

6) Add Substrate 100µl/well, mix it evenly then cover it with Adhesive plate sealer, incubate at 37 ℃ in dark for 10 minutes;

7) Add stop solution 50µl/well to stop the reaction, measure the result in 10 minutes.

8. INTERPRETATION OF RESULTS

Read the OD value with ELISA Reader at 450nm (630nm as reference).

For the assay to be valid:

Negative control (N) OD value < 0.2, and positive control (P) OD value ≥ 0.5;

Calculate method:

(Sample OD value - Negative control OD average value) / (Positive control OD average value-Negative control OD average value) = IRPC value

Results interpretation:

IRPC value < 0.4 Negative

IRPC value ≥ 0.4 Positive

9. STORAGE AND EXPIRE DATE

1) All reagents should be stored at 2-8℃. Do not freeze.

2) Shelf life is 12 months. Use all reagents before the expiry date on the kit.