Peste Des Petits Ruminants Virus Antibody ELISA Test Kit for Ovine

INSTRUCTION MANUAL

FOR PRODUCT No: LT53003AYSL

Arsh Biotech Pvt. Ltd.

308, Aggarwal City Mall, Road No.44,
Pitampura, Delhi-110034, India
Mobile: +91-98105-21400 | Fax: +91-11-42208444
info@arshbiotech.com

MANUAL VERSION 1.02

PRINCIPLE

The PPRV antibody ELISA test kit is used to detect Peste des petits ruminants virus antibodies in the serum of sheep and goat. This kit is based on competitive ELISA method using pre-coated PPRV antigens on microplate wells. When testing, diluted serum sample is added, and after incubation, if there is PPRV antibody in sample, it will combine with the pre-coated antigen. The antibody in sample thus blocks the combination of monoclonal antibody and pre-coated antigen. After discarding the uncombined enzyme conjugate by washing TMB substrate is added in micro-wells, and the blue signal by enzyme catalysis is in inverse proportion of antibody content in sample.

MATERIALS SUPPLIED WITH THIS KIT

1	PPRV Ag coated microplate	96T	7	Enzyme conjugate	7 ml
2	Negative control	0.5 ml	8	Substrate	11 ml
3	Positive control	0.3 ml	9	Stop solution	15 ml
4	Sample diluent	100 ml	10	Adhesive Plate sealer	2 pieces
5	PPRV Mab solution	6 ml	11	Instruction	1 piece
6	Washing solution 10X concentrated	100 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, separate serum by using regular method, the serum should bright and no hemolysis. Dilute test sample at 1:100 (1ul serum to 99ul 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 dissolve, 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 into room temperature before use, shake them 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 to be cross-contaminated while using.

3) Substrate and stop solution may have irritation to skin and eyes.

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

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

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

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

8) Pre-coated plates are disposable, do not repeat use.

7. TEST PROCEDURE

1) For every test, set 1 well for positive control and 2 wells for negative control;

2) Add sample diluent into each well, 20ul/well;

3) Add sample, negative control and positive control to their corresponding wells, 30ul/well. (Does not mix the tips);

4) Add PPRV Mab solution, 50ul/well, shake gently to mix it evenly, cover it with Adhesive plate sealer, incubate at 37 ℃ for 45 minutes;

5) Open the adhesive plate sealer, discard the liquid of the well, add diluted washing buffer to each well, 250ul/well, discard the liquid, repeat the above step for 4-6 times, at last flap to dry with absorbent paper;

4) Add Enzyme Conjugate, 100ul/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 5, remember at last flap to dry with the absorbent paper;

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

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

8. RESULTS JUDGEMENT

Set zero at blank control well, and read the OD value at 450nm (using 620nm or 630nm as reference filter).

For the assay to be valid:

OD value of negative control(N) > 0.6, and

$$\frac{\text{positive value}}{\text{Negative control OD average value}} < 0.3$$

Calculate method:

$$S/N = \frac{\text{Sample OD value}}{\text{Negative control OD average value}}$$

Results interpretation:

S/N >0.55 Negative.

S/N ≤0.55 Positive.

9. Storage and expire date

Store at 2~8℃ in dark, not to be frozen, expiry date: 12 months.