VERSION 1.01

PESTE DES PETITS RUMINANTS VIRUS (PPRV) ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE
By Immunomart

Peste Des Petits Ruminants virus (PPRV) Antibody ELISA test Kit for Bovine

Catalogue Number. IP100204

Principle

This kit is used to detect specific antibody against Peste des petits ruminants virus (PPRV) Antibody in serum qualitatively, for monitoring antibody after PPRV immune and serological diagnosis of infection in sheep,goat.

This kit use competition ELISA method, PPRV antigen is pre-coated on enzyme micro-well strips. When testing, add diluted serum sample and Monoclonal antibody working solution, after incubation, if there is PPR virus specific antibody, it will compete the pre-coated antigen with antibody in the working solution, discard the uncombined antibody and other components with washing; then add Enzyme Conjugate, making Enzyme react with monoclonal antibody which combine on plate, discard the uncombined enzyme conjugate with washing; Add TMB substrate in micro-wells, the blue signal by Enzyme catalysis is in inverse proportion of antibody content in sample, use ELISA reader at 450nm wave-length to measure the absorbance A value in reaction wells after adding stop solution to stop the reaction.

Specifications: 96 wells × 2.

Components

	Code item	**Spec.**
1	PPRV Antigen Coated plates 96 wells	1/2 plate
2	Enzyme Conjugate	11/22 ml
3	10X Washing buffer	100 ml
4	Substrate solution	11/22 ml
5	Sample dilution	100 ml
6	Monoclonal antibody working solution	6/12 ml
7	Stop solution	15 ml
8	Negative control	800 ul
9	Positive control	400 ul
10	Adhesive Foil	2/4 pieces
11	Instruction	1 piece

Material required not provided

1. Micropipettes: 0.5μL~10μL、10μL~100μL、100μL~1000μL
2. Disposable pipette tips
3. Measuring cylinder: 500 ml
4. 96 wells microplate reader

5. Distilled water or deionized water
6. Bottle or microplate washing machine

Sample preparation

Take animal whole blood, get serum by using regular method, the serum should bright and no hemolysis

Washing buffer preparation

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

Operation procedures

1). for each test, set 1 well for positive control, 2 wells for negative control.
2) Add Sample dilution: 30ul/well, into each well; then add negative control, positive control, serum sample, each 20ul/well into it's corresponding well, at last add Monoclonal antibody working solution: 50ul/well into each well, mix gently(do not mix use tips);
3) Cover it with Adhesive Foil; incubate at 37 ℃ for 45 minutes;
4) Open the adhesive foil, discard the liquid of the well, add diluted washing buffer to each well, 250ul/well, then discard the liquid, repeat the above step for 5 times, at last flap to dry with the absorbent paper;
5) Adding Enzyme Conjugate, 100ul/well, Cover it with Adhesive Foil, incubate at 37 ℃ for 30 minutes;
6) Open the adhesive foil, discard the liquid of the well, washing for 5 times as step 4), remember at last flap to dry with the absorbent paper;
7) Add substrate solution,100ul/well, mix it evenly then cover it with Adhesive Foil, incubate at 37 ℃ in dark for 10 minutes;
8) Add stop solution 50ul/well to stop the reaction, measure the result in 10 minutes

Results judgement

Read the OD value with ELISA Reader at 450nm (630nm as reference).
For the assay to be valid:
Negative control (N) OD value> 0.6,
OD value of positive control (P) /average OD value of Negative control (N) < 0.3;

Calculate method:

Sample OD value/average OD value of Negative control (N)= S/N value

Results interpretation

S/N value>0.7: Negative
S/N value≤0.7: Positive

Notes

1) *Return all reagents into room temperature before use, put all reagents at room temperature for at least 1 hour. 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 been polluted when using.
3) Substrate and stop solution may have irritation to skin and eyes, be careful to use.
4) Do not expose Substrate to strong light and avoid contact with the oxidant.
5) PPRV-Ag 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 can get the best results. Pipetting operation, timing, and washing of the whole process must be precise.
8) The antigen coated plate is disposable, do not repeat use.

storage: store at 2-8℃, dark, sealed, dry place, no frozen..
Expiry date: 12 months; date of production is on box.