Peste Des Petits Ruminants Virus (PPRV) Antibody ELISA

Enzyme Immunoassay for the estimation of Peste Des Petits Ruminants Virus (PPRV) Antibody ELISA

REF : KINE7075

Ver 2.0

For Veterinary Use Only

Σ 1 x 96 wells

Store at 2 - 8 ºC

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Introduction:

The Kinetic Biotech ELISA kits use monoclonal antibodies and recombinant antigens for consistent and reliable results.

Intended Use:

The Sheep Peste Des Petits Ruminants Virus (PPRV) Antibody ELISA is used as an analytical tool for qualitative determination in serum and plasma.

Principle:

The method employs competitive enzyme-linked immunosorbent assay (ELISA) technique to assay the level of Sheep Peste Des Petits Ruminants Virus (PPRV) Antibody in samples. Standards or Samples competes with the Antibody Solution, to form a complex with the Sheep Peste Des Petits Ruminants Virus (PPRV) Antigen coated microtiter well. Wells are washed to remove the excess conjugate and Streptavidin:HRP Conjugate is added to the microplate and incubated. After incubation and a washing step TMB Substrate, are added. Blue color develops on incubation and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the Sheep Peste Des Petits Ruminants Virus (PPRV) Antibody in the samples is inversely proportional to the yellow color developed (absorbance) in the wells.

Materials Provided:

1. Sheep PPRV Antigen Coated Microtiter Plate – 1 x 96 wells
2. Negative Control – 1 ml
3. Positive Control – 1 ml
4. HRP Conjugate – 11 ml
5. Antibody Solution – 6 ml
6. (20X) Wash Buffer – 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Timer.
6. Absorbent Paper.

Handling/Storage:

1. It is advisable to aliquot and store the HRP Conjugate concentrated at -20°C upon receipt. Rest of the kit components should be stored at 2-8°C. Immediately discard any excess working reagents after running your assay.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Veterinary Use Only.

Sample Preparation and Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to 2ºC-8ºC. Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.

Preparation Before Use:
Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Sample preparation:

Add 10ul of sample and to this add 40ul of wash buffer, mix well with gently shaking. Samples should be loaded onto the bottom without touching the well wall.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 25 ml of 20X Wash Buffer in 475 ml of DI water.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of the protein. High Dose Hook Effect is due to excess of protein present in the sample. To avoid the same, dilute the samples to be assayed with a compatible diluent.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of total Sheep Peste Des Petits Ruminants Virus (PPRV) Antibody antibody.
4. It is recommended that all Controls and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at 37°C.
2. Pipette out **50 ul** of **Controls** or **Samples** in each well.
3. Add **50 ul of Antibody Solution** to all wells. Cover with adhesive membrane and Incubate at 37°C for 30 minutes in dark.
4. Aspirate and wash plate 5 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells.
5. Add **100 ul** of **HRP detection conjugate** into the respective wells.
6. Incubate at 37°C for 30 minutes in dark.
7. Repeat step no. 5.
8. Add **100 ul** of **TMB Substrate** in each well.
9. Incubate the plate at 37°C for 15-20 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
10. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
11. Read the absorbance at 450 nm with a microplate reader.

Validity of the test:

The test is valid if the following conditions are met:

Under normal experimental conditions, the A value of the negative control should be ≥1.0, and the A value of the positive control should be ≤ 50% of the A value of the negative control.

Interpretation of Results:

1. PI (Percentage Inhibition%) = $\frac{(1 - A_S)}{A^{NC}} \times 100\%$

If PI is ≥ 50%, it is considered positive; if PI is < 50%, it is considered negative.

A_S - the A value of the sample;
A_{NC} - the average A value of negative controls.

2. When the results of thi s experiment are negative, it indicates that the antibody levels in goat/sheep is insufficient. It is recommended to administer the corresponding vaccine as a supplementation.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Safety Precautions:

- **This kit is For Veterinary Use Only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (<0.1% w/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from **animal body fluids** or organs used in the preparation of this kit were tested and found negative for viral antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed

- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

- In any case GLP (Good Laboratory Practice) should be applied with all general and individual regulations to the use of this kit.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Results
1A 2A	Blank Blank			
1B 2B	Positive Control Positive Control			
1C 2C	Negative Control Negative Control			
1D 2D	Sample Sample			
1E 2E	Sample Sample			
1F 2F	Sample Sample			
1G 2G	Sample Sample			
1H 2H	Sample Sample			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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