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LT018001BZ2

ELISA KIT FOR PPRV ANTIBODY

Research Use Only

For *in vitro* applications - not for consumption

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VERSION 1.2B

1.INTENDED USE

The kit is used to detect PPRV (Peste des Petits Ruminants Virus) Antibody in serum and plasma of sheep and goat qualitatively, evaluate the immune status of Peste des petits ruminants vaccine and assist in serological diagnosis of infected animals.

2.KIT CONTENTS

1.PPRV antigen coated microplate [96TX2]
2.Enzyme conjugate [12ml]
3.Sample Dilution [25ml]
4.PPRV-IgG Negative control serum [1ml]
5.PPRV-IgG Positive control serum [1ml]
6.Substrate [12mlX2]
7.Stop Solution [12ml]
8.10X Washing Buffer [50ml]
9.Adhesive Foil [2pcs]
10.User manual [1pc]

Storage and Expiry date: Store at 2~8℃ in dark, no freezing, expiry date: 12 months.

3.MATERIALS REQUIRED NOT PROVIDED

Pipettes and Tips, ELISA Reader, Eppendorf tubes, Absorbent paper, Distilled Water, and 37°C Incubator.

4.SUMMARY AND PRINCIPLE

This kit is based on Competitive Enzymatic Immunoassay method. PPRV antigen is pre-coated on micro-well plate. When testing, add diluted serum sample and Enzyme conjugate. After incubation, if there is PPR virus specific antibody in the sample, it will bind to the PPRV antigen coated on the plate and prevent the enzyme-labeled monoclonal antibody from binding to the antigen on the plate. Conversely, if the sample does not contain PPRV-specific antibody, it will not bind to the antigen coated on the plate. After washing to remove the unbound antibody and other components, add substrate to the microwells to form a blue product through enzymatic catalysis. After adding stop solution to terminate the reaction, use a microplate reader at 450nm/630nm double wavelength to measure the absorbance value.

5.SAMPLE COLLECTION

Serum - Allow the serum to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 20 minutes. Collect the supernatants carefully.
If sediments occur during storage, centrifugation should be performed again.

Plasma - In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as anti coagulant. Add EDTA or sodium citrate and mix them for 10-20 minutes. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. If sediments occur during storage, centrifugation should be performed again.

Note:
Samples should be centrifuged adequately and no hemolysis or granule is to be allowed.

6.WASHING BUFFER PREPARATION

Bring 10X Concentrated washing buffer into room temperature before use. If there are salt crystals, put it in a at 37 ℃ water bath for 5~10min to dissolve it, then dilute it with deionized water 10 times (for example, to prepare 200ml washing buffer: 180ml of deionized water + 20ml of 10X concentrated washing buffer, mix it evenly). The diluted washing buffer can be stored at 4℃ for about a week.

7.NOTE

1) Bring all reagents to room temperature before use. Shake it evenly before use.

2) Do not mix used reagents from different kits and different lot no. while using.

3) Substrate and stop solution may cause irritation to skin and eyes, be careful.

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 Micro-well plate into dry foil bag and seal to put it back at 2-8 ℃.

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

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.PROCEDURE

1) Take the pre-coated microplate (according to the number of samples, it can be disassembled and used separately), set 2 wells for positive control, 2 wells for negative control, add 50μl positive control serum or negative control serum to it's well accordingly; others are sample wells.

2)Firstly add 40μl sample dilution, then add 10μl sample into each well. At last, add 50μl Enzyme conjugate to each well, shake gently to mix it evenly, cover the plate with adhesive foil, incubate at 37 ℃ for 30 minutes;

3) Open the adhesive foil, discard the liquid of the well, add diluted washing buffer to each well, 300ul/well, be static for 30 seconds, then discard the liquid,. Repeat this step for 5 times. At last tap on a absorbent paper stack to dry;

4) Add substrate solution, 100ul/well, mix evenly then cover it with Adhesive Foil. Incubate at 37 ℃ in dark for 15 minutes;

5) Add stop solution 50ul/well to stop the reaction, measure the result in 10 minutes. Read the OD value with ELISA Reader at 450nm (630nm as reference).

9. VALIDITY OF ASSAY

As the test is based on Competitive Enzymatic Immunoassay, for the assay to be valid:

OD value of Negative control (N) ≥1.0,
OD value of positive control (P) ≤0.3;

10.CALCULATION AND RESULT INTERPRETATION

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

If,
S/N value≥0.5: Negative
S/N value<0.5: Positive

11.TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

Still if you have any concerns please feel free to write to us at customerservice@lifetechindia.com

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