PPRV Antibody Competitive ELISA Kit

PPRV Ab cELISA

Product Number: E40151-1
Product Unit: 1 plate-96wells, 2 plates-192wells, 5 plates-480wells,
for 10plates, 2 units of 5plates will be provided

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1. Introduction

Peste des petits ruminants (PPR) is a viral disease, caused by a morbillivirus closely relatives of domesticated small ruminants, as well as camels. It is characterized by severe morbidity and mortality rates, and has a high economic impact in areas of Africa, the Middle East, and Asia, where small ruminants contribute to guaranteeing livelihoods. Affected animals present high fever and depression, along with eye and nose discharges.

This kit utilizes antibody of PPRV, based on which competitive ELISA or lateral flow immunoassay were further developed for laboratory testing purpose.

2. Description of Test

The current PPRV Antibody Competitive ELISA kit is designed to detect PPRV antibody in goat serum samples. The 96 well microtiter plate was precoated with recombinant nucleocapsid protein (protein N). During testing, samples are added into the microplate wells, in which the precoated antigen will capture the PPRV antibody in sample and formed antigen-antibody complex. None specific antibody is discarded by a washing step. Then goat-anti-swine IgG conjugated with horseradish peroxidase (HRP) is added into each well, compete with antibody in the sample. After another washing step to remove unreacted conjugate, substrate is added and a blue color will be developed if PPRV antibody is present. The enzyme reaction is stopped and the OD450nm value is measured. The measured intensity is negatively proportional to the amount of antibody present in the sample.

This ELISA kit can be used to detect PPRV antibody in serum of **goat, sheep, etc**.

3. Precautions

- Store the kit at 2-8℃,Check the lot number and expiration date before use.
- Bring the test kit to room temperature before use. For example, take it out from the cold storage and put at room temperature for at least 30min.
- The stop solution in the kit is acidic, please make sure do not touch it with your hand or skin.
- The component of the kit is noninfectious, but the field sample shall be treated as potentially infectious. Please handle all these materials properly according to your lab regulations.
- After experiment, all lab materials shall be handled properly according to local regulations.

4. Limitations of Test

This ELISA kit is currently designed for veterinary use. We recommend validating in your own lab with different methodologies to confirm the performance. If it is not used for the mentioned purpose, please contact us for help.

5. Reagent Provided

The kit contains the following items.

Item No.	Description	1 plate	2 plates	5 plates
1	Microplate pre-coated with antigen	1 X 96 wells	2 X 96 wells	5 X 96 wells
2	Positive Control	1 X 1 ml	1 X 1 ml	2 X 1 ml
3	Negative Control	1 X 1 ml	1 X 1 ml	2 X 1 ml
4	Enzyme Conjugate	1 X 25 ml	1 X 25 ml	3 X 25 ml
5	TMB Substrate	1 X 25 ml	1 X 25 ml	3 X 25 ml
6	Stop Solution	1 X 20 ml	1 X 20 ml	2 X 20 ml
7	25X Wash Buffer	1 X 30 ml	1 X 30 ml	3 X 30 ml
8	Sample Buffer	1 X 30 ml	1 X 30 ml	3 X 30 ml
9	Kit Instruction	1set	1set	1set

6. Instrument Required

- ELISA reader with 450nm
- Micropipette 20-200ul
- Micropipette Multi-Channel 50-300ul

7. Reagent Preparation

- Make sure the kit and all test samples are returned to room temperature (18-26°C) before use. Shake each reagent gently before adding into the well.
- The positive control, negative control, enzyme conjugate, serum buffer, substrate and stop solution can be used directly.
- Wash buffer (25X): dilute the 25X wash buffer provided in the kit with deionized water in the volume ratio of 1:24. For example, 10ml 25X wash buffer + 240ml deionized water. The diluted wash buffer can be stored at 2-8°C for 7 days.
- Sample preparation: centrifuge the blood sample at 4000rpm for 10min, then collect the supernate. Then diluent the sample in the ratio of 1:9 with the help of serum diluent plate (eg, 15ul of serum sample + 135ul of sample buffer).

8. Assay Procedure

1. Take the microplate from the zip-bag, and mark the location of the sample.
2. **Add 100ul negative control** into two wells (100ul/well).
3. **Add 100ul positive control** into another two wells (100ul/well).
4. **Add 100ul diluted serum sample** into microwells (100ul/well). The sucker needs to be replaced when different samples are drawn.
5. Cover the plate with plate cover and incubate at **25°C for 45min**.
6. Pour the liquid out from the wells and wash with wash buffer **(300ul per well) for 5 times**. Tap the residue liquid against absorbent paper to make sure the plate is dry after washing.
7. **Add 100ul of HRP enzyme conjugate** into each well. Cover the plate again and then incubate at **25 °C again for 30min**.
8. Repeat the washing step again.
9. **Add the substrate** into each well, **100ul per well**. Cover the plate again and then incubate at **25 °C again for 10min**. note: avoid direct sunlight.
10. **Add 50ul stop solution** into each well to stop the reaction.
11. Using ELISA reader to read the plate at 450nm.

9. Result Determination

Test result is valid when "the Average OD value of negative control" >=0.5, "IN of positive control" > 50%. Otherwise, please run the analysis again with new kit.

1) Calculation of IN:

$$\frac{\textbf{Mean OD of Negative Control – Mean OD of Sample}}{\textbf{Mean OD of Negative Control}} \textbf{ x 100% = IN\%}$$

2) Criteria of Positive and Negative results.

Positive: **IN% ≥50%**; **Negative**: **IN% < 50%**

10. Storage and expiration

The kit shall be store at 2-8°C, avoid direct sunlight.

The valid period is 12 months.

11. References

(1) https://www.oie.int/en/disease/peste-des-petits-ruminants/

(2) https://www.msdvetmanual.com/generalized-conditions/peste-des-petits-ruminants/overview-of-peste-des-petits-ruminants