Peste des petits ruminants Virus (PPRV) Antibody GENLISA™ ELISA

REF : KAD1109

Ver1.0

RUO

Immunoassay for qualitative screening of Peste des petits ruminants Virus (PPRV) Antibody indirect levels in serum or plasma for ovine.

RUO	**For Research Use Only**	REF	**Catalog Number**
	**Store At**	LOT	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF **KAD1109** Σ **96 tests**

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Introduction:

The Peste des petits ruminants Virus (PPRV) Antibody ELISA kit is used to test Peste des petits ruminants Virus (PPRV) Antibody in serum. Peste des petits ruminants (PPR) is also known as 'goat plague', 'Kata', 'syndrome of stomatitis-pneumoenteritis' or 'ovine rinderpest'. It is an important infectious viral disease of domestic and wild small ruminants that threatens the food security and sustainable livelihood of farmers.

Intended Use:

It is used to detect PPRV antibody in serum and plasma of sheep and goat qualitatively, evaluate the immune status of Peste des petits ruminants vaccine and assisted serological diagnosis of infected animals.

Principle:

This kit use competitive ELISA method, PPRV antigen is pre-coated on enzyme micro-well strips. When testing, add diluted serum sample and Monoclonal Enzyme conjugate, after incubation, if there is PPR virus specific antibody, it will bind to the PPRV antigen on the coating 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 coating 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 double wavelength to measure the absorbance A value in the reaction well.

Materials Provided:

1. PPRV antigen coated microplate – 1 x 96 Wells
2. Enzyme Conjugate – 12 ml
3. Sample dilution – 25 ml
4. Negative control – 1 ml
5. Positive control – 1 ml
6. Wash Buffer (20X) – 2 x 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Plate sealer
10. Instruction Manual

Materials Required But Not Provided

1 Micropipette: 0.5 ul-10 ul, 10 ul-100 ul, 100 ul-1000 ul.
2 Disposable pipette tips.
3 Graduate: 500 ml.
4 Microplate Reader: 96 wells with 450nm wavelength.
5 Distilled water or deionized water.
6 Microplate Washer

Storage and expire date

1 The samples are chicken serum, which should be collected with no bacteria. The storage time should be less than 1 week at 2-8℃, if for long term, it should be kept at -20℃.
2 Avoid use of the samples with severe hemolysis, precipitate, contaminated by bacteria or protein suspension.

Health Hazard Warnings:

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

Reagent Preparation

1. Return 20 X Concentrated washing buffer into room temperature before use, If there is salt crystals, put it in at 37℃water bath for 5~10min to dissolve it, then dilute it with deionized water at 10 times. The diluted washing buffer can be stored at 4℃for about a week.

2. To make Wash Buffer (1X); dilute 25 ml of 20X Wash Buffer in 475 ml of DI water.
3. Serum Sample should be diluted 1:100 on dilution plate for e.g. 1µl Sample Serum + 99µl Sample Diluent.

Sample preparation

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

Procedural Notes

1. Return all reagents into room temperature before use, put all reagents at room temperature for at least 30 minutes. 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.

Assay Procedure:

1. Bring all reagents to Room Temperature prior to use. It is strongly recommended that all **Controls and Samples** should be run in duplicates.

2. Set 2 wells for positive control and 2 wells for negative control add **50 ul** Controls into the respective wells. add **40 uL** sample dilution into each well.

Note -Negative control and Positive control do not need dilute. Exchange tip after taking sample every time, record the situation of the sample on plate accurately. Shake the sample evenly before adding it.

3. Add **10 ul** sample in each well.

4. Cover the plate with sealer & incubate at **37°C** for **30 minutes**.

5. Aspirate and wash plate 5 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly or alternatively a microtiter plate or strip washer may be used.

6. Add **50 ul** of **Enzyme Conjugate** to the wells.

7. Cover the plate with sealer & incubate at **37°C** for **30 minutes**.

8. Add **100 ul** of **TMB Substrate** solution into each well.

9. Cover the plate with sealer & Incubate at **37°C** for **10 minutes** in dark

10. Add **100 ul** of **Stop Solution** to each well.

11. Read the absorbance at 450nm in a microplate reader within 10 minutes.

Validity of the test:

The test is valid if the following conditions are met,

Mean Absorbance of Negative Control ≥ 1.0
Mean Absorbance of Positive Control ≤ 0.3

Results interpretation

NC - means Negative control's OD 450nm value
PC - means Positive control's OD 450nm value

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

S/N value ≥ 0.5 = Negative

S/N value < 0.5 = Positive

Safety Precautions:

- **This kit is For Research 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/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from Bovine/Pig fluids or organs used in the preparation of this kit. Therefore, handle all components and all patient 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 should be applied with all general and individual regulations to the use of this kit.

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