Technical Manual

Porcine Pseudorabies Virus Antibodies ELISA Kit

- Catalogue Code: ADES0006
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This kit is comprised by HRP conjugate, other reagents and ELISA Microtiter plate pre-coated with recombinant Porcine Pseudorabies Virus (PRV) gD protein. Apply the principle of enzyme-linked immunoassay (ELISA) to detect PRV-Ab in serum, plasma of porcine. During the experiment, add control and samples into the ELISA Microtiter plate, PRV-Ab will be bound with the antigen on the ELISA Microtiter plate. Then wash the plate to remove unbound components, horseradish peroxidase (HRP) conjugate is added to each ELISA Microtiter plate well. The unbound HRP Conjugate will be removed by washing and substrate reagent is added for color development. At last, end the reaction by adding Stop Solution to produce a yellow product. There is a positive correlation between the OD value of samples and the concentration of PRV-Ab. Measure the absorbance value of each well by using a microplate reader with 450 nm (630 nm) wavelength, then we can judge whether PRV antibody exist in the sample.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Dilution plate	96 wells
HRP Conjugate	11 mL
Sample Diluent	50 mL
20×Concentrated Wash Buffer	40 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	1 mL
Negative Control	1 mL
Plate sealer	3 piece
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Experimental instrument

- Microplate Reader with 450nm wavelength filter or dual-wavelength (450/630nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C incubator or water bath
- Deionized or distilled water

- Absorbent paper

4. Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
3. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. A void the kit to strong light.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh.
7. The results shall depend on the readings of the microplate reader.
8. **Each reagent is optimized for use in the ADES0006. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ADES0006 with different lot numbers.**
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

5. Storage and expiry date

Store at 2-8° C. Avoid freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the packing box.

6. Reagent preparation

1. Use the conventional method to prepare serum or plasma, the serum or plasma must be clear, no hemolysis and no pollution. Samples can be conserved at 2~8° C in 1 week, and it should be stored at - 20° C for a long term storage.
2. **Diluted serum/plasma**: Dilute the sample serum or plasma with the **Sample Diluent** at 1:39 (5 μL sample serum or plasma and 195 μL of sample diluent, mix fully). The positive/negative control do not need to be diluted.
3. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

7. Assay procedure

Restore all reagents and samples to room temperature (25° C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8° C.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 1 well for blank control and 2 wells for negative/positive control respectively. **Samples need test in duplicate (**Blank well is not needed when using dual wavelength 450 nm/630 nm for detection).
2. **Add sample:** add 100 µL of **Sample Diluent** to the blank control well, add 100 µL of **positive/negative control** to positive/negative control well, and add 100 µL of **Diluted serum/plasma** to the sample wells.
3. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37° C for 30 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 300 µL of **Wash Buffer** to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** add 100 µL of **HRP Conjugate** into each well (except the blank control well), cover the plate sealer and incubate at 37° C for 30 min in shading light.
6. **Wash:** repeat step 4 for washing.
7. **Color Development:** add 50 µL of **Substrate Reagent A** and 50 µL of **Substrate Reagent B** into each well. Cover the plate sealer and mix thoroughly, incubate at 37° C for 10 min in shading light.
8. **Stop reaction:** add 50 µL of **Stop Solution** into each well, mix thoroughly.
9. **OD Measurement:** adjusted zero with the blank control, measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) wavelength. Blank well is not needed when using dual wavelength 450 nm/630 nm for detection.

8. Reference value

Normally, the OD of negative control $\leqslant$ 0.3 and the OD of positive control $\geqslant$ 0.6.

9. Interpretation of the results

$$S/P = \frac{\text{average absorbance of sample} - \text{average absorbance of negative control}}{\text{average absorbance of positive control} - \text{average absorbance of negative control}}$$

1. Positive result: S/P $\geqslant$ 0.2
2. Negative result: S/P < 0.2

3. Unimmunized animal: positive result indicates that it may be infected with PRV.
   Immunized animal: The antibody levels at the time of the sample were monitored and recorded, and the distribution of antibody levels and the trend of immune status of the flock were analyzed based on the results.

10. Limitations of this test method

1. This test is only used as the qualitative detection of PRV antibody in serum and plasma of porcine. A rough estimate (high, general, low) of the concentration of this antibody can be concluded according to the OD values.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

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