Porcine Pseudorabies Virus IgE Antibody Distinguishing Test Kit  
Cat# KBVE113

1. Intended Use
Aujeszky’s disease or pseudorabies is caused by a herpes virus and affects mainly pigs which are the only known reservoir of the disease. It is an important disease of pigs causing severe economic losses. The Pseudorabies virus IgE antibody ELISA test kit can be used to detect anti PRV-IgE antibody in the serum and distinguish naturally infected virus from vaccinated swine.

2. Introduction
The PRV IgE ELISA test kit is made from the antigen coated microplate, enzyme conjugate (HRP goat-anti-pig IgG) and other reagents. It applies the indirect ELISA principle to test the antibody against PRV-IgE antibodies in the serum.

3. Principle
The test kit is the anti IgE coated microplate made through the protein expressed by pseudorabies virus IgE gene. In the test, the diluted control serum and sample are added, then incubate. If PRV-IgE specific antibodies exist in the sample, it will be bound with the antigens of the microplate. Then the unbound antibodies and other components are removed by washing. Next, add IgG-HRP to specifically bind with the compound of antibody and antigen on the microplate. The unbound conjugation will be removed by washing, add the TMB substrate in the well, react with HRP to a blue product. At last, end the reaction by adding stop solution.

4. Kit Contents
1. Microtitre well plate, 2x96 strip wells
2. IgE-IgG Negative Control, 1.5ml
3. IgE-IgG Positive Control, 1.5ml
4. Enzyme Conjugate, 22ml
5. Substrate A, 12ml
6. Enzyme Conjugate, 22ml
7. Sample Diluent, 25ml
8. Stop Solution, 12ml
9. 20XWash Buffer, 50ml

5. Material Required But not Provided
1. Microplate Reader(single-wave length: 630 nm).
4. Constant temperature box or incubator.
5. Oscillator.

6. Sample requirement
1. The samples are porcine serum, which should be collected with no bacteria. The storage time should be less than 1 week at 2-8 °C, if for long term, it should be kept at -20°C.
2. Avoid to use the samples with severe hemolysis, precipitate, contaminated by bacteria or protein suspension.
3. The EDTA, heparin sodiun and other anticoagulants will not affect the results.

7. Preparation of Washing Solution
Washing solution: Return the Wash Concentrate (20×) into room temperature (about 25°C) before use, shake to dissolve the precipitated salt (Better warm it at 37°C water for 5~10 min), then dilute Wash Concentrate (20×) solution with distilled water or deionized water at 20 times, the diluted washing solution can be stored at 2~8°C for 7 days.

8. Test Procedure
1. Take out the pre-coated microtiter plate(just to the required quantity). Set 1 blank control well (Add nothing); Set 2 wells for negative control serum, add undiluted negative control serum, 100 μL/well; 2 wells for positive control serum, add undiluted positive control serum, 100 μL/well. Others are sample wells, dilute the sample at 50 times with the sample diluent solution, then add the diluted sample into sample wells, 100 μL each.
2. Mix gently by shaking the plate manually, incubate at 37 °C for 30 min.
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3. Pour the liquid out of the wells, add washing solution to each well, 300 μL/well, static for 1 min. Repeat 5 times, then pat to dry on absorbent paper.

4. Add 100 μL enzyme conjugate in each well, except blank control well, and incubate at 37 °C for 30 min.

5. Repeat step 2(washing).

6. Add 50 μL substrate A(one drop) and 50 μL substrate B(one drop) into each well, mix gently by shaking the plate manually, incubate for 10 min at 25 °C in the dark.

7. Add 50 μL stop solution (one drop) in each well, and measure the result within 10 min.

8. Measure the A value with a photometer at 630 nm, set zero for the blank well, and read A value of each well.
   (Note: if haven’t set zero at blank control well before reading, then the result should minus A value of blank control well)

9. Results
   • Generally speaking, the PRV-Positive control OD value should be ≥0.6, the PRV-Negative control OD value should be less than 0.1.
   • Results:
     If the OD630nm value≥0.55, it is judged to be positive; from 0.5 to 0.55, doubtful; and if less than 0.5, negative.

10. Interpretation of the result
   1. Severe hemolysis, fiber protein in the serum separation is not sufficient, containing erythrocytes, a precipitate, a sample with bacteria may lead to false positive.
   2. Negative results may occur on individual pigs after vaccines due to individual differences or immune duration.
   3. Positive results for serological diagnosis and epidemiologic investigation of swine to be combined with other methods and clinical data.

11. Product performance
   1. Specificity: to test 30 negative control serums, no false positive.
   2. Sensitivity: to test 30 positive control serums, no negative.
   3. Precision: CV (%) no bigIgEr than 15% (n=10)
   4. Stability: Store at 2~8℃ for 6 months or store at 37℃ for 3 days, the result can reach the above 3 standard.

12. Precautions and warnings for users
   1. This test kit is suitable for in vitro diagnostics.
   2. Wear glove and working clothes when operate, treat the test kit as containing infectious material.
   3. Experiment rubbish should be dealt with high pressure steam sterilization at 121 ℃ for 30 minutes, or treated with 5.0g/L sodium hypochlorite disinfectant for 30 minutes, then discard.
   4. MicroWell plate removed from the refrigerated environment should be balanced moisture to dry at room temperature, then can be opened. Put back unused MicroWell plate into dry foil bag and sealed at 4 ℃. Unused liquid reagent should cover caps, store at 2-8 ℃ in dark with other group components.
   5. If the 20×concentrated washing buffer appears crystal, it is normal, put at 37℃ until been dissolved.
   6. Should use Micropipettor to add sample and reagents, and often proof its accuracy.
   7. When adding washing buffer, should be full but no overflow, avoid appearing free enzyme at mouth of well or cross pollution between wells.
   8. Stop solution is corrosive, use large amount of water to wash immediately when touch the skin or clothes.

Specifications: 96 wells × 2

expiry date: 6 months from date of production.

Storage: Store at 2-8 ℃, don't expose in strong light.

Production Date: See outer-packing of the test kit.
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