Porcine Japanese B Encephalitis Virus Ab ELISA Kit

Cat# KBVE116

1. Intended Use
The porcine encephalitis virus ELISA test kit is used for the detection of the porcine encephalitis virus antibodies in swine serum; assessment the immunity conditions against porcine encephalitis virus in the pig farms and investigation of the epidemiology of the porcine encephalitis virus.

2. Principle
The kit is based on an indirect enzymatic immunoassay (Indirect ELISA). The antigen is coated on plates. When a sample serum contains specific antibodies against virus, they will bind to the antigen on plates. Wash the unbound antibodies and other components. Then add a specific peroxidase conjugate (IgG-HRP). After incubation and washing, add the TMB substrate. A colorimetric reaction will appear, measured by a spectrophotometer (630 nm).

3. Kit Contents
1. PCV Microtitre well plate, 2x96 strip wells
2. Negative Control Serum, 1.5ml
3. Positive Control Serum, 1.5ml
4. Enzyme Conjugate, 22ml
5. Substrate A, 12ml
6. Substrate B, 12ml
7. Enzyme Conjugate, 22ml
8. Sample Diluent, 50 ml
9. Stop Solution, 12ml
10. 20XWash Buffer, 50ml

4. Materials Required But Not Provided
1. Microplate Reader (Single wave-length: 630 nm).
4. Constant temperature box or water bath box.
5. Oscillator.

5. 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 sodium and other anticoagulants will not affect the results.

6. Test Procedure
1. Take out the coated plates (Can be detached) and record the sample position on a worksheet. Set one blank control well, add nothing; Set 2 wells for negative control serum, add undiluted negative control serum, 2 wells for positive control serum, add undiluted positive control serum, 100 μL/well. Others are sample wells, dilute the sample at 40 times with the sample diluent (such as add 6μL sample into 234μL sample diluent) and add to the wells, 100 μL each.
2. Mix gently, incubate at 37°C for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add Washing buffer (dilute the 20×concentrated washing buffer at 20 times with deionized water) into each well fully, stand for 3 min. Repeat 3 times, at last time pat to dry on absorbent paper.
4. Add 100 μL enzyme conjugate into each well (except the blank well).
5. Cover plate with new adhesive foil. Incubate at 37 °C for 30 min.
6. Repeat step 3 (washing).
7. Add substrate A one drop (50 μL) and substrate B one drop (50 μL) into each well, mix properly, incubate for 10 min at room temperature (25 °C) in the dark with new adhesive foil.
8. Add stop solution one drop (50 μL) into each well, mix gently and determine the result within 10 min.
9. Measure the A value of each well with a photometer at wave length 630 nm, set zero for the blank control well. Note: if haven’t set zero at blank control well before reading, then the result should minus A value of blank control well.
7. Results
For the assay to be valid, the positive control wells’ A value must be greater than or equal to 0.40, and the negative control wells’ A value is less than 0.20. Otherwise the test is invalid, need test again. If the sample’s A value is greater than 0.4, it is judged to be positive; from 0.2 to 0.4, doubtful; and if less than 0.2, negative. (Note: if haven’t set zero at blank control well before reading, then all the above result should minus A value of blank control well.)

8. Reference
The healthy pigs not being natural infected or immune swine JEV disease virus vaccine is negative, A value is less than 0.2. For piglet, because its body contains maternal antibody, its determination A value may be greater than critical value.

9. 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 epidemiological investigation of swine to be combined with other methods and clinical data.

10. 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 bigger 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.

11. 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 per kit.
Expiry date: 6 months from date of production..
Storage: Store at 2~8℃, in the dark.
Production Date: On outer-packing of the test kit.
**LIMITED WARRANTY**

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

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