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Porcine Japanese B Encephalitis Virus (JEV) antibody ELISA test kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT80003AYSL (192 Tests)

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MANUAL VERSION 1.01

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INTRODUCTION

The Porcine Japanese B Encephalitis Virus IgG Antibody ELISA test kit is used for the detection of the porcine encephalitis virus IgG antibodies in swine serum; assessment the immunity conditions against porcine encephalitis virus, serological diagnosis of pig infection in the pig farms and investigation of the epidemiology of the porcine encephalitis virus.

PRINCIPLE

This kit is based on solid-phase enzyme-linked immunosorbent assay (ELISA) principle composed by the antigen coated microtiter plate (coated with JEV antigen) and other reagents. In this reaction the coated antigen binds specifically to form complex of coated antigen+JEV-IgG+enzyme labeled anti-pig-IgG antibody on the microplate. With the TMB substrate, it generates color. The depth of color is relative with the content of the JEV-IgG, when the value of color is above threshold, the pigs are vaccinated well or have natural infection.

MATERIASL SUPPLIED WITH THIS KIT

1	JEV antigen coated microplate	96T ×2	6	Substrate	12 ml ×2 orange lid
2	Enzyme conjugate	22 ml yellow lid	7	Stop solution	12 ml blue lid
3	Sample diluent	50 ml transparent lid	8	20×concentrated washing buffer	50 ml white lid
4	JEV-IgG Negative control serum	1.5 ml green lid	9	Adhesive Foil	2 pieces
5	JEV-IgG Positive control serum	1.5 ml red lid	10	Instruction	1 piece

MATERIALS REQUIRED BUT NOT PROVIDED

1) Microplate Reader (double-wave length: 450/630 nm).

2) Precise micropipette (single-channel 1-100µl, 0.5-10µl, multi-channel 30-300µl).

3) Constant temperature box or water bath.

4) Oscillator.

5) Microplate Washer.

6) Disposable tips (10µl, 200µl).

7) Deionized water.

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 ℃, if for long term, it should be kept at -20℃.

2 Avoid to use the samples with severe hemolysis, precipitate, contaminated by bacteria or protein suspension.

PREPARATION

1. Bring ELISA reagents to the room temperature (20-25 ℃) for 30 min to get best results.

2. Sample dilution: dilute the sample 40 times (eg. 5µl serum sample + 195µl sample diluent solution). Mix the diluted sample evenly to get better results.

3. Washing solution preparation: Dilute the 20X concentrated washing buffer with deionized water at 20 times. (eg.50ml 20X concentrated washing buffer + 950ml deionized water), if there is crystallization in the 20×concentrated washing buffer, it is normal, dissolve it at 37℃.

PROCEDURE

1. Take out the coated plates (strips can be detached) and record the sample position on a worksheet. Set 2 wells for negative control serum, add undiluted negative control serum, 2 wells for positive control serum, add undiluted positive control serum, 100µl per well. Other wells can be used as sample wells, add the diluted sample, 100µl per well.
2. Mix gently, cover and incubate at 37℃ for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add the diluted Washing buffer into each well fully, soak for 10s and pour out. Repeat 3 times, at last time pat to dry on absorbent paper.
4. Add 100µl enzyme conjugate into each well.
5. Cover plate with new adhesive foil. Mix gently, Incubate at 37 ℃ for 30 min.
6. Repeat step 3(washing).
7. Add substrate 100µl into each well, mix properly, incubate for 10 min at 37 ℃ in the dark with new adhesive foil.
8. Add stop solution 50µl into each well, mix gently and determine the result.
9. Measure the OD value of each well with a photometer at 450nm (with 620 or 630nm as reference filter).

RESULTS

For the assay to be valid, the positive control wells average OD value must be greater than or equal to 0.6, and the negative control wells' average OD value is less than 0.1. Otherwise the test is invalid, and you need to test again.

The result is judged by S/P value:

S/P = (Sample OD- $\overline{NCx}$)/($\overline{PCx}$- $\overline{NCx}$),

where $\overline{NCx}$ means Negative control's average OD value(calculate as 0.05 for value lower than 0.05),

$\overline{PCx}$ means Positive control's average OD value

If S/P≥0.25, it is positive; less than 0.25, it is negative.

PRECAUTIONS AND WARNINGS FOR USERS

1. Read the Instruction carefully before you run the test.

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 kept 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 have caps covered, store at 2-8 ℃ in dark with other group components.

5. Should use Micropipettor to add sample and reagents.

6. When adding washing buffer, it should be full but should not overflow, avoid pipetting free enzyme at mouth of well or cross contamination between wells.

7. Stop solution is corrosive, use large amount of water to wash immediately when touch the skin or clothes.

Specifications: 96 wells × 6.

Expiry date: 12 months.

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