Porcine Japanese Encephalitis Virus Antibody (JEV Ab) ELISA Kit

Technical Manual

(ELISA)

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Product Information

Intended Use

Porcine Japanese encephalitis is a mosquito-borne, acute zoonotic infectious disease caused by the Porcine Japanese Encephalitis Virus (JEV). Infected pigs primarily exhibit symptoms such as abortions and stillbirths in pregnant sows, and testicular swelling in boars.

This assay is designed to detect antibodies against Japanese Encephalitis Virus (JEV) in porcine serum or plasma. It can be used to evaluate the immunological efficacy of the JEV vaccine.

Principle and Application

This kit comprises a coated Microtiter Plate with recombinant E2 protein of porcine JEV, HRP conjugate, and other accompanying reagents. It employs the principle of enzyme-linked immunosorbent assay (ELISA) to detect antibodies against JEV in the porcine serum or plasma. During the experiment, control serum and test sample are added to the plate. After incubation, if the sample contains JEV antibodies, they will bind to the antigens coated on the microtiter plate. Following washing steps to remove unbound components, the HRP conjugate is added, which specifically binds to the antigen-antibody complexes on the plate. After washing again to remove unbound HRP conjugate, substrate reagents are added to the wells and react with the enzyme-labeled complexes, resulting in a blue color. The intensity of the color is directly proportional to the amount of specific antibody present in the sample. The reaction is then terminated by adding a stop solution, turning the solution yellow. The absorbance of each well is measured at a wavelength of 450nm using a microtiter plate reader (microplate reader) to determine the presence of antibodies against JEV in the sample.

Composition of the Kit

Reagent	Specification		
Microtiter Plate	96wells	96wells×2	96wells×5
Sample Diluent (yellow cap)	1×50mL	1×50mL	1×200mL
HRP conjugate (red cap)	1×11mL	2×11mL	2×26mL
Concentrated Wash Buffer (20×) (white cap)	1×40mL	1×40mL	1×200mL
Substrate Reagent A (white cap)	1×6mL	1×11mL	1×26mL
Substrate Reagent B (black cap)	1×6mL	1×11mL (brown cap)	1×26mL
Stop Solution (yellow cap)	1×6mL	1×11mL	1×26mL
Positive Control (red cap)	1×1.0mL	1×1.5mL	1×2.0mL
Negative Control (green cap)	1×1.0mL	1×1.5mL	1×2.0mL
Adhesive Membrane	1	2	5
Sealed bag	1	1	2
Dilution plate	1	2	5
Instruction	1	1	1

Storage conditions

The kit shall be stored at 2-8 °C. Avoid moisture.

Shelf life: 12 months. Please use within 2 months after opening. The date of manufacture is presented in the label of the box.

Test Apparatus Required but Not Supplied

microplate reader, adjustable micropipette, constant temperature device (37°C), centrifuge.

Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

1.Sample Preparation: The serum/plasma should be clear, without hemolysis or contamination. Samples can be stored at 2-8°C for up to 1 week, and for long-term storage, they should be kept at -20°C.

2.Sample dilution: Dilute the prepared sample 40-fold using the sample diluent (e.g., add 195µL of sample diluent to the dilution plate, followed by the addition of 5µL of the sample, and mix well). What obtained is the diluted sample. Negative and positive controls do not require dilution.

3.Solution preparation: Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19). What obtained is the working wash buffer.

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

ELISA procedure

Place all reagents and samples to room temperature

(adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

1.Put the required number of the wells on the plate and set up 2 wells each for negative/positive control.

2.Add 100μL of **negative control** to each negative control well. Then add 100μL of **positive control** to each positive control well. For each sample well, add 100μL of the **diluted sample.**

3.Shake the plate gently by hand (or use a microplate shaker) for 5s, cover it with adhesive membrane and incubate at 37°C (water bath recommended) in the dark for 30 minutes.

4.Discard the liquid from the wells. Add 350μL of **working wash buffer** to each well, let stand for 30 seconds, then discard. Repeat the washing process 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).

5.Add 100μL of **HRP conjugate** to each well, cover with adhesive membrane, and incubate at 37°C in the dark for 30 minutes.

6.Washing. Same as step **4.**

7.First, add 50μL of **substrate reagent A** to each well, followed by 50μL of **substrate reagent B.** shake gently by hand (or use a microplate shaker) for 5s, cover with adhesive membrane, and incubate at 37°C in the dark for 10 minutes.

8.Add 50μL of stop solution to each well and shake gently by hand (or use a microplate shaker) for 5s. Read absorbance (Optical Density; **OD**) at 450nm with microplate reader (with 630nm as a reference wavelength). Finish this step within 10min.

Reference Value

The average OD value of the negative control(OD_{NC}), should be ≤0.3, while the average OD value of the positive control(OD_{PC}), should be ≥0.6.

Interpretation of Test Results

1. $SP=\frac{OD_S-OD_{NC}}{OD_{PC}-OD_{NC}}$

If SP is ≥0.2, it is considered positive; if SP is <0.2, it is considered negative.

OD_S—the OD value of the sample;

2. When the results of this experiment are negative, it indicates that the antibody levels in pigs are insufficient. It is recommended to administer the corresponding vaccine as a supplementation.

Limitations of the Test Method

This experiment serves only as a qualitative detection of antibodies against Japanese Encephalitis Virus in pig serum or plasma. The rough assessment of antibody levels as strong, moderate, or weak can be made based on the high or low values of the SP value.

Attention

1. During the experiment, gloves and lab coats should be worn. Strict and comprehensive disinfection and isolation protocols should be followed. All experimental waste should be treated as infectious material.

2. The stop solution is corrosive. Avoid contact with skin and clothing. If accidentally contacted, rinse immediately with a large amount of tap water.

3. When taking the microtiter plate out of a refrigerated environment, it should be brought to room temperature before opening the bag. Unused microplate wells should be stored in the sealed bag with a desiccant.

4. During washing, each well should be filled completely with liquid to prevent any residual enzyme on the well's rim from remaining unwashed.

5. The samples used for testing should be kept fresh.

6. The determination of test results must be based on the readings from the microplate reader.

7. Components from different lot numbers must not be mixed.