Porcine Japanese Encephalitis Virus antibody ELISA Kit

Catalog No: E-AD-E067

96T

Version Number:	V1.1
Replace version:	V1.0
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This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
Email: techsupport@elabscience.com
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

Apply the principle of enzyme-linked immunoassay (ELISA) to detect Japanese Encephalitis Virus antibody (JEV-Ab) in serum of porcine. During the experiment, add control and samples into the ELISA Microtiter plate, JEV-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 JEV-Ab. Measure the absorbance value of each well by using a microplate reader with 450 nm (630 nm) wavelength, then we can judge whether JEV antibody exist in the sample.

Kit components

Item	Specification
ELISA Microtiter plate	96 wells
Dilution plate	96 wells
HRP Conjugate	10 mL
Sample Diluent	25 mL
20×Concentrated Wash Buffer	25 mL
Substrate Reagent A	5 mL
Substrate Reagent B	5 mL
Stop Solution	5 mL
Positive Control	0.5 mL
Negative Control	0.5 mL
Plate Sealer	1 piece
Sealed Bag	1 piece
Manual	1 copy

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

Other materials required but not supplied

Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)

High-precision transferpettor, EP tubes and disposable pipette tips

37°C incubator or water bath

Deionized or distilled water

Absorbent paper

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. Avoid th e 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 E-AD-E067. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E067 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.

Storage and expiry date

Store at 2-8℃. Avoid freeze.

Please store the opened plate at 2-8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample preparation

1. **Serum:** Use the conventional method to prepare serum, the serum must be clear, no hemolysis and no pollution. Samples can be conserved at 2-8℃ in 1 week, and it should be stored at - 20℃ for a long term storage.
2. **Diluted serum:** Dilute the sample with the **Sample Diluent** at 1:39 (5 μL sample and 195 μL of Sample Diluent, mix fully).
3. **Diluted Positive/Negative Control :** Dilute the **Positive/Negative Control** with the **Sample Diluent** at 1:3 (60 μL of Positive/Negative Control and 180 μL of Sample Diluent, mix fully).
4. **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.

Assay procedure

Restore all reagents and samples to room temperature 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℃.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 2 wells for negative/positive control respectively. **Samples need test in duplicate.**
2. **Wash:** add 300 μL of **Wash Buffer** to each well and wash.Wash procedure for 1 times. 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)
3. **Add sample:** add 100 μL of **Diluted Positive/Negative Control** to positive/negative control well, and add 100 μL of **Diluted serum** to the sample wells.
4. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37℃ for 30 min in shading light.
5. **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. 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).
6. **HRP conjugate:** add 100 μL of **HRP Conjugate** into each well, cover the plate sealer and incubate at 37℃ for 30 min in shading light.
7. **Wash:** repeat step 5 for washing.
8. **Color Development:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** into each well. (Add **Substrate Reagent A** first and then **Substrate Reagent B**, please add in order) Cover the plate sealer and mix thoroughly, incubate at 20-25℃ for 10 min in shading light.
9. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
10. **OD Measurement:** measure the absorbance value (A-value) of each well by using a Microplate Reader with 630 nm wavelength. This step should be finished in 10 min after stop reaction.

Reference value

Normally, The experimental data are considered valid when the average $OD_{Positive\ control} \geq 0.80$, the average $OD_{Negative\ control} < 0.20$.

Interpretation of the results

S/P = $OD_{Sample} / OD_{Positive\ control}$

1. Positive result: $S/P \geq 0.21$;
2. Negative result : $S/P < 0.21$.
3. Unimmunized animal: positive result indicates that it may be infected with JEV.
   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.

Limitations of this test method

1. This test is only used as the qualitative detection of JEV antibodies in serum of porcine. A rough estimate of antibody concentration (high, general, low) can be calculated based on the **S/P**.
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.