

GENERAL DESCRIPTION

Japanese encephalitis virus (JEV) is a Culex mosquito-borne, zoonotic flavivirus causing encephalitis in humans and reproductive disorders such as abortion and stillbirth in swine. The virus in endemic areas is persisting in nature through a cycle of transmission involving mosquitoes, domestic and wild birds, domestic pigs, and humans. Swine is the main natural amplifier and reservoir of JEV. World Health Organization recommends having a strong prevention and control program in all regions where the disease is a public health concern, utilizing efficient surveillance tools and reporting mechanisms (WHO Fact Sheet No 386, 2015). This test kit as a surveillance tool was designed to detect JEV-specific antibodies in swine serum as an evidence of infection or vaccination. The serum antibodies bind to specific antigen coated on the wells of the polystyrene plate. The JEV antibodies bound to the antigen are specifically reacted with a horseradish peroxidase (HRPO)-labeled anti-swine IgG conjugate. The complex of JEV antibody and anti-swine IgG-HRPO conjugate is detected by the addition of enzyme substrate and colorimetric measurement of subsequent color development. Strong color development indicates the presence of antibodies to JE virus in the sample serum. Very weak or no color development indicates the absence of antibodies to JEV in the sample serum.

KIT COMPONENTS

| Reagents | 192 tests | 480 tests |
|---------------------------------|-----------|-----------|
| ① JEV Antigen Coated Plate | 2 plates | 5 plates |
| ② 10X Washing Buffer | 120mLX1 | 240mLX1 |
| ③ Dilution Buffer | 120mLX1 | 240mLX1 |
| ④ HRPO Anti-Swine IgG Conjugate | 40mLX1 | 70mLX1 |
| ⑤ Positive Control, PC | 3.0mLX1 | 7.0mLX1 |
| ⑥ Negative Control, NC | 3.0mLX1 | 7.0mLX1 |
| ⑦ TMB Substrate | 30mLX1 | 70mLX1 |
| ⑧ Stop Solution | 20mLX1 | 40mLX1 |
| ⑨ Sealing Film | 2 sheets | 5 sheets |
| ⑩ Instruction Manual | 1 copy | 1 copy |

PREPARATION

- All reagents must be allowed to come to room temperature (20~25°C) before use. Mix reagents by gentle swirling. After use, return to 2~8°C.
- 1X washing buffer preparation
 - Shake 10X Washing Buffer(②) gently.
 - Dilute 1 part of 10X Washing Buffer(②) with 9 parts of deionized water. The diluted 1X washing buffer is stable for 2 weeks at room temperature (20~25°C).

- Serum dilution
 - Use fresh serum samples for the best result. Serum samples can be stored at 2~8°C for less than 3 days or -20°C for a longer period. Do not freeze and thaw serum samples repeatedly. **Sera with hemolysis or bacterial contamination are not suitable for the analysis!**
 - Visible solid materials in serum samples should be separated by centrifugation.
 - Prepare 1mL deep-well-plate (DWP, 96-well, not offered) or suitable tubes.
 - Dilute 5μL of test serum with 495μL of Dilution Buffer (③) in a DWP well or a suitable tube.
- Do not dilute the Positive Control (PC, ⑤) and the Negative Control (NC, ⑥).**
- TMB Substrate (⑦) should be warmed up for 30 minutes at room temperature (20~25°C) before use (10mL/plate). If stored at low temperature, the color development may be poor.

TEST PROCEDURE

- Remove the Antigen Coated Plate (①) from protective foil pouch.**
- Add 100μL of the diluted serum sample to each well of the plate, keeping the wells A1 and A2 for Positive Control (PC, ⑤) and the wells B1 and B2 for Negative Controls (NC, ⑥). **Use care not to spill samples from well to well.**
- Add 100 μL of undiluted Positive (PC, ⑤) and Negative (NC, ⑥) Controls in the designated wells.
- Seal the plate with enclosed Sealing Film (⑨).
- Incubate the plate for one hour (±2 min) at 37°C.
- Wash the plate three times with 300 μL of 1X washing buffer. Get rid of moisture by tapping the plate on a dry paper towel.
- Add 100 μL of HRPO Anti-Swine IgG Conjugate (ready for use, ④) to each well.
- Seal the plate with enclosed Sealing Film (⑨).
- Incubate the plate for one hour (±2 min) at 37°C.
- Wash the plate as described in Step 6.
- Add 100μL of TMB Substrate (⑦) to each well.
- Cover the plate with enclosed Sealing Film (⑨).
- Incubate the plate for 10 minutes (±1 min) at room temperature (20~25°C). Protect the plate from direct light exposure.
- Add 50μL of Stop Solution (⑧) to each well of the plate. Shake the test plate shortly (5~10 sec.). **Be careful not to spill.**
- Measure and record the A (450nm) for samples and controls immediately.
- Validate and calculate the results.

Plate template example (1-well Test)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|----|----|----|----|----|----|----|----|----|----|----|----|
| A | PC | PC | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 | 77 | 85 |
| B | NC | NC | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 | 78 | 86 |
| C | 1 | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 | 79 | 87 |
| D | 2 | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 | 88 |
| E | 3 | 9 | 17 | 25 | 33 | 41 | 49 | 57 | 65 | 73 | 81 | 89 |
| F | 4 | 10 | 18 | 26 | 34 | 42 | 50 | 58 | 66 | 74 | 82 | 90 |
| G | 5 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 | 75 | 83 | 91 |
| H | 6 | 12 | 20 | 28 | 36 | 44 | 52 | 60 | 68 | 76 | 84 | 92 |

RESULT INTERPRETATION

1. Validate if the mean OD of the PC is higher than 0.500 and the mean OD of the NC is lower than 0.200. If these criteria are not met, the test are invalid and the samples must be retested.
2. Calculate a S/P ratio by dividing the mean OD value of a sample by the mean OD value of the PC as below.

$$S/P = \frac{\text{Sample OD}}{\text{PC OD}}$$

3. Result interpretation
 - 1) Test samples having ≥ 0.25 S/P are positive.
 - 2) Test samples having < 0.25 S/P are negative.

| S/P value | Interpretation |
|-----------------|----------------|
| $S/P \geq 0.25$ | Positive |
| $S/P < 0.25$ | Negative |

4. Example of result calculation and interpretation
 - 1) ODs of PC : 1.121, 1.201
Mean OD = $(1.121 + 1.201) / 2 = 1.161$ (valid)
 - 2) ODs of NC : 0.085, 0.091
Mean OD = $(0.085 + 0.091) / 2 = 0.088$ (valid)
 - 3) OD of Sample : 0.870
S/P ratio of the sample = $(0.870 / 1.161) = 0.749$
 - 4) Result interpretation: Positive

PRECAUTIONS

1. All reagents must be allowed to come to room temperature (20~25°C) before use. Mix reagents by gentle swirling. After use, return to 2~8°C.
2. Read this instruction manual thoroughly and follow all steps strictly for successful use of the product.
3. All test samples should be considered potentially infectious and all items contacting the samples should be considered contaminated.
4. Do not use expired or contaminated reagents.
5. Do not use reagents from other kits or lots.
6. Do not mix reagents from different lots of this same product.
7. Do not expose the reagents to excessive heat or direct light during storage and incubation.
8. Incomplete washing adversely may affect the result and precision of the assay.
9. Avoid microbial contamination of the reagents.
10. Avoid contamination of the TMB Substrate(7) with the HRPO Anti-Swine IgG Conjugate(4).
11. Wear personal protective equipment (PPE) such as lab coat, goggle, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.

12. Do not eat, drink, smoke or apply cosmetics where kit reagents are handled. Do not pipette by mouth.
13. Pipette tips must be changed after each pipetting step. Use a clean disposable pipette tip for all steps.
14. Use care not to spill samples from well to well.
15. Deionized water or equal must be used to prepare the washing buffer.
16. Unused strips should be stored in the sealed foil pouch at 2~8°C. Re-sealed strips are recommended to use within one week.
17. For veterinary use only.

STORAGE AND STABILITY

Store all reagents at 2~8°C. Do not freeze. Reagents remain stable until the expiration date when stored as instructed.

REFERENCES

| Test | Results |
|---|--|
| Analytical Sensitivity (Limit of Detection) | - 1:10 at HI - 1:10 at PRNT - 1:8 at SNT |
| Analytical Specificity (Cross Reactivity) | No cross reaction against antibodies to PPV, PRRSV, EMCV, ADV, and other swine pathogens |
| Diagnostic Sensitivity | - 95.3% sensitivity vs HI - 98.7% sensitivity vs PRNT - 98.7% sensitivity vs SNT |
| Diagnostic Specificity | - 95.3% specificity vs HI - 95.0% specificity vs PRNT - 95.0% specificity vs SNT |

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QUICK PROTOCOL

