GENLISA™ Porcine Japanese Encephalitis Virus Antibodies ELISA

REF : KAD1016

Ver2.2

RUO

Immunoassay for Qualitative screening of Porcine Japanese Encephalitis Virus levels in porcine serum, plasma and other body fluid

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF KAD1016 Σ 2 x 96 tests

KRISHGEN BioSystems

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Introduction:

Japanese encephalitis (JE) virus is a member of the family Flaviviridae, genus Flavivirus. The enveloped virus is closely related to the West Nile virus and the St. Louis encephalitis virus. The positive sense single-stranded RNA genome is packaged in the capsid which is formed by the capsid protein. The outer envelope is formed by envelope protein and is the protective antigen.

Intended Use:

The GENLISA™ Porcine Japanese 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:

The GENLISA™ Porcine Japanese Encephalitis Virus IgG antibody ELISA employs the qualitative enzyme immunoassay technique. Japanese Encephalitis Antigen is pre-coated onto microwells. Samples and controls are pipetted into microwells and antibodies to Japanese Encephalitis present in the sample and control are bound by the capture antigen. Then, a HRP (horseradish peroxidase) conjugated Anti-Porcine IgG is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Anti- Japanese Encephalitis in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm

Materials Provided:

1. JEV antigen Microtitre coated plate – 2 x 96 Tests
2. Anti-Porcine IgG:HRP Conjugate – 22 ml
3. Negative control serum – 1.5 ml
4. Positive control serum – 1.5 ml
5. (1X) Sample diluent – 50 ml
6. (20X) Wash Buffer– 2 x 25 ml
7. TMB Substrate – 2 x 12 ml
8. Stop Solution – 2 x 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Precise micropipette (single-channel 1-100 ul, 0.5-10 ul, multi-channel 30-300 ul).
3. Deionized (DI) water.
4. Constant temperature box or water bath box.
5. Oscillator.
6. Disposable tips (10 ul, 200 ul)

Handling/Storage:

1. Reconstitute or dilute only the specific reagents mentioned in the reagent preparation section, when ready to run the assay.
2. Store all kit components at 2ºC to 8ºC when not in use and do not expose them to temperatures greater than 37ºC or less than 2ºC

3. Do not use kit components after the expiration date.
4. Do not repeatedly freeze/thaw the reagents as loss of activity may result.
5. Before using, bring all components to Room Temperature (18-25ºC). Upon assay completion return all components to appropriate storage conditions.
6. ELISA plate pouches contain desiccant. Keep the plates sealed in the pouch with desiccant in the refrigerator when not in use.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. Handle Stop Solution carefully. Obtain medical attention in case of accidental ingestion of kit components.
3. Avoid assay of samples containing Sodium azide as it is hazardous.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. The kit cannot test samples which contain NaN_3, because NaN_3 inhibits HRP activity.
2. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
3. **Serum**- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.
4. **Plasma**- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 20-min at the 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.
   Note: Grossly hemolyzed samples are not suitable for use in this assay.
5. Dilute the serum with the sample diluent in a ratio of 1:40 for eg. 5 ul sample and 195 ul of sample diluent
   Note: do not dilute the positive and negative control

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 25 ml of (20X) Wash Buffer in 475 ml of DI water.

Procedural Notes:

1. Read all the instructions thoroughly before performing the test.
2. Allow all reagents to reach Room Temperature before beginning and reconstitute or dilute the required reagents.
3. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess unreacted reagents is essential.
4. All Controls and Samples should be assayed at least in duplicates.
5. The assay has been optimized to be used with the protocol mentioned. Any deviation from the same may invalidate the results.

Assay Procedure:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. All steps must be performed at 37°C.

2. Pipette **100 ul of Positive and Negative Controls** and diluted **Samples** into the respective wells.

3. Cover the plate and incubate for 30 minutes at 37ºC.

4. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

5. Add **100 ul of Anti-Porcine IgG:HRP Conjugate** into each well.

6. Cover the plate and incubate for 30 minutes at 37ºC.

7. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

8. Add **100 ul of TMB Substrate** in each well.

9. Incubate the plate at 37ºC for 10 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

10. Pipette out **100 ul of Stop Solution**. Wells should turn from blue to yellow in color.

11. Read the absorbance at 450 nm with a microplate reader.

Interpretation of Results:

NC = Negative Control OD at 450nm
PC = Positive Control OD at 450nm

$$\text{Cut-Off Factor} = \frac{\text{Absorbance Value of Sample - Absorbance of Negative Control}}{\text{Absorbance of Positive Control - Absorbance of Negative Control}}$$

Positive Samples: Cut-Off Factor ≥ 0.25
Negative Samples: Cut-Off Factor < 0.25

Validity of the test:

The test is valid if the following conditions are met,
Mean Absorbance of Negative Control < 0.4
Mean Absorbance of Positive Control ≥ 0.6

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

Sensitivity:
The kit is based on capture antigen which is highly specific for porcine Japanese Encephalitis Virus.

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools.

While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
#1	<10%	<10%
#2	<5%	<5%

Safety Precautions:

- **This kit is for research use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from porcine and other animal body fluids or organs used in the preparation of this kit were tested and found negative for few viral antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

Typical Example of a Work List

Well #	Contents	Absorbance at 450 nm	Mean Absorbance	Cut-Off Interpretation
1A 2A	Negative Control Negative Control			
1B 2B	Positive Control Positive Control			
1C 2C	Sample Sample			
1D 2D	Sample Sample			
1E 2E	Sample Sample			
1F 2F	Sample Sample			
1G 2G	Sample Sample			
1H 2H	Sample Sample			

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