GENLISA® Japanese Encephalitis (JE) Antigen ELISA

REF : KBVH350

Ver 1.2

RUO

Enzyme Immunoassay for the Quantitative Determination of Japanese Encephalitis Protein in human serum and plasma

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 KBVH350 Σ 96 tests

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Introduction:

Japanese encephalitis virus (JEV) is an enveloped, single-stranded RNA virus which belongs to the genus Flavivirus within the family Flaviviridae. JEV occurs as a single serotype but has considerable antigenic variation. Scientists have been isolated more than 50 strains in Japan. PCR, RT-LAMP, ELISA, and immunochromatography are the diagnostic methods currently used to detect JEV.

Intended Use:

The GENLISA® Japanese Encephalitis (JE) Antigen ELISA is used as an analytical tool for quantitative determination of JEV Envelope Protein in human serum and plasma.

Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. JEV Envelope Antibody is pre-coated onto microwells. Samples and standards are pipetted into microwells and JEV Envelope Protein present in the sample are bound by the capture antibody.Then a HRP (horseradish peroxidase) conjugated Japanese Encephalitis Virus Antibody 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 JEV Envelope Protein in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Japanese Encephalitis Envelope Antibody Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Japanese Encephalitis Envelope Antibody.	96 wells
JEV Envelope Protein Standard concentrated	Recombinant Japanese Encephalitis Envelope Protein (concentrated 690 ug/ml- 10 ul)	1 vial
Japanese Encephalitis Virus Antibody:HRP concentrated	Japanese Encephalitis Virus Antibody conjugated to Horseradish Peroxidase (concentrated – 1 mg/ml – 10ul)	1 vial
(1X) Standard Diluent	Buffered protein base with 1:1000 dilution human serum and preservatives 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane.	10 ml
Detection Diluent	Buffered protein base with preservative 0.02% methylisothiazolone and 0.02% bromonitrodioxane	12 ml
(1X) Sample Diluent	Buffered protein base with preservative 0.02% methylisothiazolone and 0.02% bromonitrodioxane	2 x 50 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized Chromogen	12 ml
Stop Solution	0.73M Phosphoric Acid	12 ml
Instruction Manual		1 no.

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. It is advisable to aliquot and store the JEV Envelope Protein standard concentrated and Japanese Encephalitis Virus Antibody:HRP concentrated at -20°C upon receipt. Rest of the kit components should be stored at 2-8°C. Immediately discard any excess Working solutions after running your assay.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-24ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Test Sample preparation - Serum and plasma samples have to be diluted 1:1000 (v/v), e.g. for 1:1000 (1 ul sample + 999 ul sample diluent) prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires the samples to be kept at -20°C.

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.
4. **Standards Preparation**: Thaw the concentrated standard vial. Prepare the Standards as per the below table. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
690 ug/ml	Original standard	Original standard
4000 ng/ml	Standard No.7	5.8 ul Original standard + 994.2 ul Standard Diluent
2000 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent
1000 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent
500 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent
250 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent
125 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
62.5 ng/ml	Standard No.1	500 ul Standard No.2 + 500 ul Standard Diluent
0 ng/ml	Standard No.0	Only Standard Diluent

5. **Working Japanese Encephalitis Virus Antibody:HRP Conjugate – Refer to the Reagent Preparation sheet attached with the IFU and COA (enclosed in the kit).**

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.

2. High Dose Hook Effect may be observed in samples with very high concentrations of Japenese Encephalitis antigen. High Dose Hook Effect is due to excess of antibody for very high concentrations of Japenese Encephalitis antigen present in the sample.
3. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent.
   Thus if the Japenese Encephalitis antigen concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Japenese Encephalitis antigen.
5. It is recommended that all Standards and Samples be assayed in duplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at 37°C.

2. Add **100 ul** of **prepared Standards** or **Samples** into the respective wells.

3. Cover the plate and incubate for 120 minutes at 37°C.

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 **Working Japanese Encephalitis Virus Antibody:HRP Conjugate** into each well.

6. Cover the plate and incubate for 120 minutes at 37°C.

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 37°C for 30 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.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Japenese Encephalitis antigen, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Japenese Encephalitis antigen Concentration. If samples were diluted, multiply by the appropriate dilution factor.

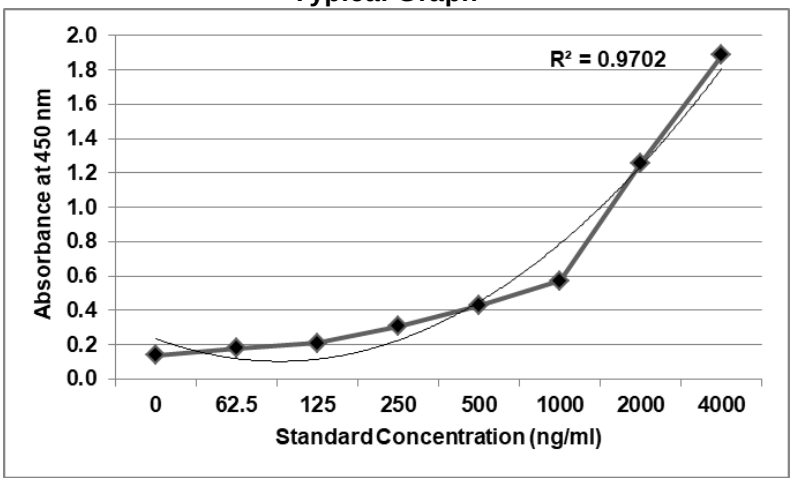
Software which is able to generate a cubic spline curve-fit or a polynomial curve (2nd order) is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 4000 ng/ml standard.

Typical Data

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.14	--	--
62.5	0.179	68.9	110.2
125	0.211	113	90.4
250	0.308	261.9	104.8
500	0.428	489	97.8
1000	0.574	1008.6	100.9
2000	1.259	1995	99.8
4000	1.885	4001.8	100

Typical Graph



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:

This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

Sensitivity:
Limit of Quantification: It is defined as the lowest concentration of an analyte that can be determined with an acceptable repeatability and the LOQ was found to be 60 ng/ml.
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2*SD.
10 replicates of '0' standards were evaluated and the LOD was found to be less than 62.5 ng/ml.

Specificity:
The kit utilizes a highly specific recombinant antigen of Japanese Encephalitis Virus produced using recombinant DNA technology in a suitable expression system. The antigen corresponds to immunodominant regions of the JEV envelope (E) protein (~50–55 kDa), which is the primary target of host immune responses.

The ELISA coating antibody is a rabbit monoclonal antibody with the immunogen being recombinant fragment of Japanese encephalitis virus Envelope. (strain: Jaoars982).

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 with low (62.5 ng/ml), medium (500 ng/ml) and high (4000 ng/ml) concentrations. 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
Low	<10%	<10%
Medium	<5%	<5%
High	<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 human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV 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.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

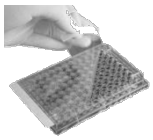
Components (2-8ºC) → 30 mins RT Before Use → 

Thaw at Room Temperature (18-24ºC) → USE NOW

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

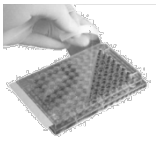
3.  Pipette **100 ul Standards** / **Samples** into the respective wells.

4. Cover plate  and incubate for 

at 37°C.

5.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

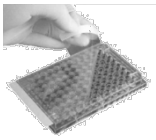
6.  Pipette **100 ul Working Japanese Encephalitis Envelope Virus Antibody:HRP Conjugate** into each well.

7. Cover plate  and incubate for 

at 37°C.

8.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

9.  Pipette **100 ul TMB Substrate** into each well.

10. Cover plate  and incubate for 

at 37°C.

11.  Pipette **100 ul Stop Solution** into each well.

12. Read absorbance at 450 nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml Japenese Encephalitis antigen equivalent
1A 2A	zero std. zero std.			
1B 2B	62.5 ug/ml 62.5 ug/ml			
1C 2C	125 ng/ml 125 ng/ml			
1D 2D	250 ng/ml 250 ng/ml			
1E 2E	500 ng/ml 500 ng/ml			
1F 2F	1000 ng/ml 1000 ng/ml			
1G 2G	2000 ng/ml 2000 ng/ml			
1H 2H	4000 ng/ml 4000 ng/ml			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

LIMITED WARRANTY

Krishgen Biosystems Private Limited does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems Private Limited shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems Private Limited shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems Private Limited be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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THANK YOU FOR USING A KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	**Japanese Encephalitis Envelope Antibody Coated Microtiter Plate (96 wells)**
STD	**JEV Envelope Protein, Standard (concentrated)**
HRP CONJ	**Japanese Encephalitis Virus Antibody:HRP (concentrated)**
DET DIL	**Detection Diluent**
1X SAMP DIL	**(1X) Sample Diluent**
1X STD DIL	**(1X) Standard Diluent**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiry Date**
	**Storage Temperature**