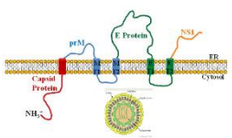
INTENDED USE

The **Human Anti-Japanese Encephalitis Virus Prm Protein (JEV-PrM) IgG** ELISA Kit is an immunoassay suitable for detecting and quantifying IgG antibody activity specific for **JEV** envelope protein E in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use.
This immunoassay is suitable for:

- Determining **immune status** relative to non-immune controls;
- Assessing efficacy of **vaccines**, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and standardizing vaccine batches & protocols

The kit contains NO virus or viral proteins. The assay is for research use only (RUO) and is not intended nor validated for diagnosing JEV. Reagents contain no virus.

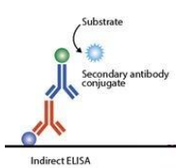
GENERAL INFORMATION



Japanese encephalitis—previously known as Japanese B encephalitis to distinguish it from von Economo's A encephalitis—is a disease caused by the mosquito-borne Japanese encephalitis virus (**JEV**). Domestic **pigs** and wild **birds** are reservoirs of the virus; transmission to humans may cause severe symptoms. One of the most important vectors of this disease is the mosquito *Culex tritaeniorhynchus*. This disease is most prevalent in Southeast Asia and the Far East. Severe rigors mark the onset of this disease in humans. Signs which develop during the acute encephalitic stage include neck rigidity, cachexia, hemiparesis, convulsions and a raised body temperature between 38 and 41oC. Mental retardation developed from this disease usually leads to coma. Japanese Encephalitis is diagnosed by detection of antibodies in serum and CSF (cerebrospinal fluid) by ELISA.

JEV is closely related to the West Nile virus and St. Louis encephalitis virus. Positive sense ssRNA genome is packaged in the capsid, formed by the capsid protein. The outer envelope is formed by **envelope (E)** protein and is the protective antigen. The genome also encodes several nonstructural proteins also (NS1-5). NS1 is produced as secretory form also. Envelope protein (E) is subsequently involved in membrane fusion between virion and host late endosomes and is synthesized as a homodimer with prM which acts as a chaperone for envelope protein E. After cleavage of prM, envelope protein E dissociates from the small envelope protein M and homodimerizes. Viral antigen can also be shown in tissues by indirect fluorescent antibody staining

PRINCIPLE OF THE TEST



The Human Anti-JEV IgG ELISA kit is based on the binding of anti-JEV IgG in samples to the JEV antigens coated on the microwells; bound antibodies are detected by specific anti-human IgG-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color is developed, which is directly proportional to the amount of antibody present in the samples. Stopping Solution is added to terminate the reaction, and absorbance at 450nm (yellow color) is then measured using an ELISA reader. The activity of anti-JEV IgG in samples is determined relative to anti-JEV-specific Calibrators.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at 4°C for long term and RT for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample/Conjugate Diluent** and store at 2-8°C until the kit lot expires or is used up.
Anti-Human IgG-HRP Conjugate Concentrate (100x) Part: H-HuG.211, 0.15ml	Anti-human IgG-HRP in buffer with detergents and antimicrobial. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
JEV prM Coated Strip Plate	910-141	8-well strips (12)	Coated with purified JEV prM antigen, and post-coated with stabilizers.
Anti-JEV Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10U/ml	910142B 910142C 910142D 910142E	0.65ml 0.65ml 0.65ml 0.65ml	Four (4) vials, each containing anti-JEV IgG; in buffer with antimicrobial.
Low NSB Sample Diluent Reduces non-specific binding	TBTm **Not** for HRP Conjugate dilution	30 ml	Buffer with protein, detergents and antimicrobial. Use as is for sample dilution. See **Assay Design**, page 3.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- ELISA reader at 450 nm and ELISA plate washer

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Caution: Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls (which have been tested non-reactive for HbsAg and Anti-HIV), and dispose of these samples and containers as biohazard waste.

Antibody Stability& Dilution

Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.

Example: Initial (1/5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1/50): **10**ul initial (1/5) + **90**ul LNSD (1/50)

Assay Design

Review Interpretation of Results (p5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **1 U/ml Calibrator**. This is usually 1/100 or greater dilution for human serum with normal levels of IgG and IgM.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Plate Set-up

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8Calibrator wells and 2 wells for each sample control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of sample diluent (blank), calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website:http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

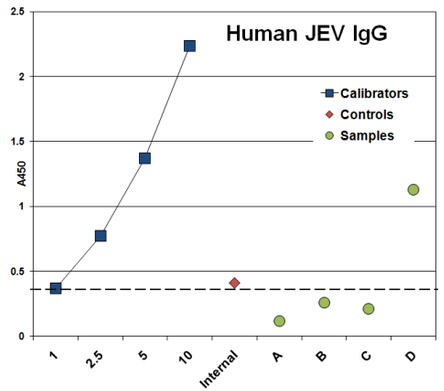
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

=Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-JEV IgG is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-JEV antiserum, derived from JEV vaccination, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or higher background negatives.

Internal Control – a true positive from a normal individual that represents the lab's experience in distinguishing low positive from negative samples. This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A, B, C,D – 3 samples (1/100) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

The 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 is **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Method B. Positive Index
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.

2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 is **Negative** for antibody.

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Example:

Experimental Samples are represented as follows:

C – Calibrator; **I** – Internal Control; **E** – Experimental sample

Results

	Assay Net OD		Calculated Antibody Activity	
Sample	Control	Exptl	Control	Exptl
1	0.325	2.281**C**	0.75	**5.29**
2	0.272	1.581**C**	0.63	**3.67**
3	0.133	0.998**C**	0.31	**2.32**
4	0.194	0.453**C**	0.45	**1.05**
5	0.289	0.767**E**	0.67	**1.78**
6	0.319	0.982**E**	0.74	**2.28**
7	0.332	1.401**I**	0.77	**3.25**
8	0.291	0.351**E**	0.68	0.81
9	0.402	0.325**E**	0.93	0.75
10	0.253	0.16**E**	0.59	0.37
Mean	0.281			
SD	0.075			
Mean +2 SD	**0.431**	= **Positive Index**		

Internal Control: Positive (>1.0) for antibody activity.
Calibrators: Ranking from 1 – 10 U/ml = 1.05 – 5.29.
Experimental: Two (2) are Positive (>1.0); 3 are Negative.

INTERPRETATION OF RESULTS (cont)

Method C. Titers from Sample Dilution Curves
The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Calibrator values can be used to normalize inter-assay values.

Calculations
1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index

= IgG Antibody Activity Units

PRODUCT SPECIFICATIONS

Specificity

Recombinant, full length, purified JEV envelope protein (E) is used to coat the microwells to detect antibodies to JEV. JEV Env protein has no significant protein sequence conservation in related ssRNA viruses such as West Nile Virus (WNV). So this kit will detect antibodies specific for JEV.

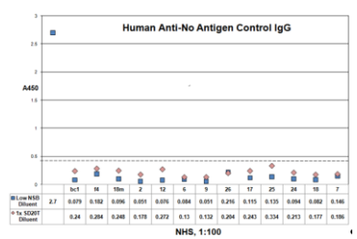
The anti-Human IgG HRP conjugate specifically detects IgG; IgM, IgA and IgE would not be detected above background.

Assay Sensitivity

The JEV-coated plate, anti-Human IgG HRP concentration, and Low NSB Sample Diluent are optimized to differentiate anti-JEV IgG from immune and non-immune individuals when samples are tested at a dilution of 1:100 or higher.

Use of Non-Antigen Coated Plates

Some sample's IgG may bind non-sepcficallfy to the plate or the non-antigen components (blockign proteins such as BSA) on the plate. Therefore, ADI provides non-antigen coated plates (Cat# 80011-SB) that are processed the same way as the coated antigen. We recommend that users test some postive samples on the plate to confirm that the binding is specific. Most samples when tested at 1:100 or higher should have A450 less than the recommended cut-off (<0.500).



Instruction Manual No. M-910-140-JEG

Human Anti-Japanese Encephalitis Virus PrM Protein (JEV-PrM) IgG

ELISA Kit #. 910-140-JEG, 96 tests

For Quantitation of Anti-JEV PrM IgG in Human Serum or Plasma or other biological fluids

For research use only, not for diagnostic or therapeutic use.

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Draft Version-Please consult the manual supplied with the kit for any lot specific change.