Version 05-06/24

User's Manual

Human Anti-Japanese Encephalitis Virus Prm Protein IgG ELISA Kit

REF **DEIAJX004**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Human Anti-Japanese Encephalitis Virus Prm Protein 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.

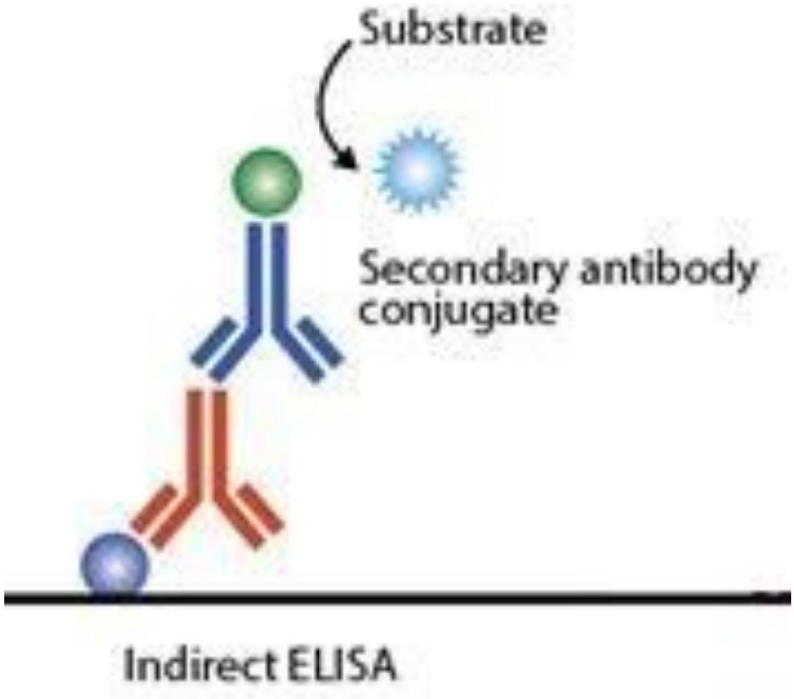
General Description

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 41°C. 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

Principles of Testing

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.



Reagents And Materials Provided

1. Wash Solution Concentrate (100x): 10ml
2. Sample Diluent Concentrate (20x): 10ml
3. Anti-Human IgG-HRP Conjugate Concentrate (100x): 0.15ml
4. JEV prM Coated Strip Plate: 8-well strips
5. Anti-JEV Calibrators: 1 U/ml 0.65ml,2.5 U/ml 0.65ml,5 U/ml 0.65ml,10U/ml 0.65ml
6. Low NSB Sample Diluent: 30 ml
7. TMB Substrate: 12 ml
8. Stop Solution: 12 ml

Materials Required But Not Supplied

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

Storage

2-8°C until the expiration date printed on the box label.

Specimen Collection And Preparation

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

Plate Preparation

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.

Reagent Preparation

1. Wash Solution Concentrate (100x):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.
2. Sample Diluent Concentrate (20x):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.
3. Anti-Human IgG-HRP Conjugate Concentrate (100x):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.

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.

Review Interpretation of Results 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.

1. 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. 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.

Calculation

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

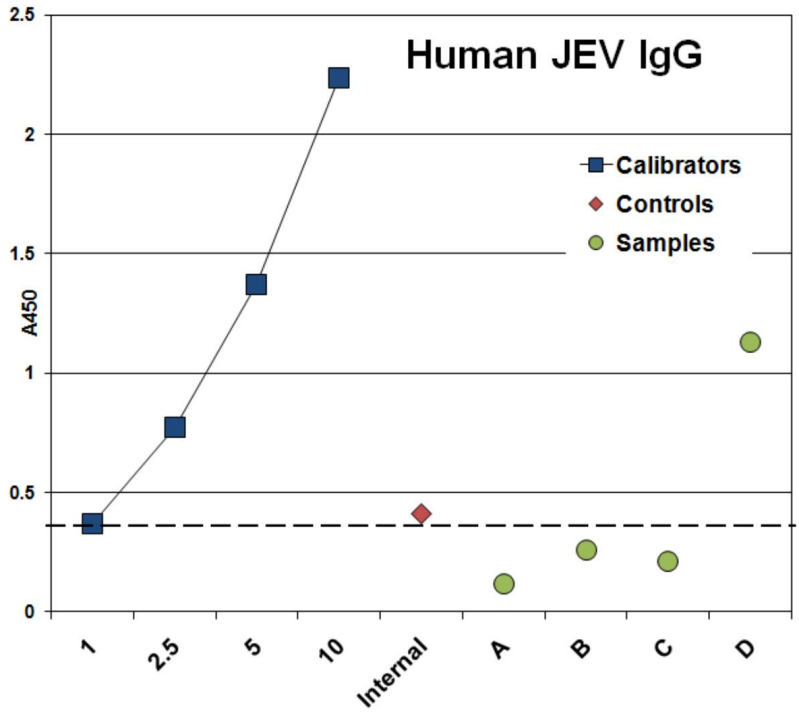
Interpretation Of Results

Method 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.

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_x0002_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 nonimmune 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

	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**		

Results

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.

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.

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.

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.

Precautions

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.