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User's Manual

Human Anti-ZIKV NS1(Zika Virus Non-structural Protein) IgG ELSIA Kit

REF **DEIAJX001**

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-ZIKV NS1(Zika Virus Non-structural Protein) IgG ELSIA Kit is an immunoassay suitable for quantifying IgG antibody activity specific for Zika Virus NS1 in serum or plasma of vaccinated, immunized and/or infected hosts.

This immunoassay is suitable for:

o Determining immune status relative to non-immune controls;

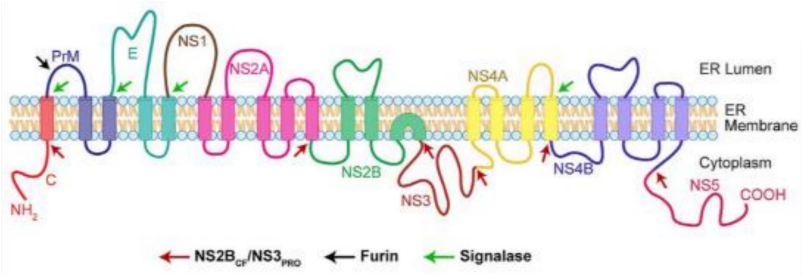
o Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;

o Qualifying and standardizing vaccine batches & protocols

The assay is for research use only (RUO) and is not intended nor validated for diagnosing Zika virus disease. Reagents contain no virus or viral antigens.

General Description

Zika virus (ZIKV), a member of the virus family Flaviviridae (flavus means yellow), transmitted by daytime-active Aedes mosquitoes, such as A. aegypti and A. albopictus. Zika virus is related to the dengue, yellow fever, Japanese encephalitis, and West Nile viruses. Like other flaviviruses, Zika virus is enveloped and icosahedral and has a non-segmented, positive-sense ss-RNA genome. There are two lineages of the Zika virus. Effective vaccines for yellow fever virus, Japanese encephalitis, and tick_x0002_borne encephalitis have been develop but there are no vaccines for Zika virus.



Zika virus genome codes for a polyprotein that is subsequently cleaved into capsid (C), precursor membrane (prM), envelope (E), and non-structural proteins (NS). The E protein composes the majority of the virion surface and is involved with aspects of replication such as host cell binding and membrane fusion. NS1, NS3, and NS5 are large, highly-conserved proteins while the NS2A, NS2B, NS4A, and NS4B proteins are smaller, hydrophobic proteins. Like other flaviviruses, both structural and non-structural protein antibodies are detected during Zika virus infection. The member of flaviviruses share 40-60% protein sequence conservation. Moreover, vaccines have become available for JEV, YFV, and Dengue. Therefore, it is important to ruLe out the presence of Zika antibodies due to vaccination and/or infection from related viruses.

Principles of Testing

The Anti-ZIKV IgG ELISA kits are based on the binding of antibodies (IgG) in samples to the recombinant, purified ZIKV antigen immobilized on the microwells. Bound antibody is detected by anti-human IgG-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color is developed by the HRP

substrate, which is directly proportional to the amount of anti-ZIKV IgG present in the sample. Stop Solution is added to terminate the reaction, and Absorbance is then measured using an ELISA reader at 450nm. The presence of antibody (IgG) in samples is determined relative to anti-ZIKV Calibrators.

Reagents And Materials Provided

1. Wash Solution Concentrate (100x): 10mL.
2. Sample Diluent Concentrate (20x): 10mL.
3. Anti-Human IgG_x005fHRP Conjugate Concentrate (100x): 0.15mL.
4. ZIKV NS1 Coated Strip Plate: 8-well strips (12).
5. Anti-ZIKV-NS1 Calibrators:. 1 U/mL 0.65 mL, 2.5 U/mL 0.65 mL, 5 U/mL 0.65 mL, 10 U/mL 0.65 mL.
6. Anti-ZIKVNS1 Positive Control: 0.65mL.
7. Low NSB Sample Diluent (LNSD): 30mL.
8. TMB Substrate: 12mL.
9. Stop Solution: 12mL.

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100uL and 1-10mL.
2. Disposable glass or plastic 5-15mL tubes for diluting samples and Anti-Human IgG HRP Concentrate.
3. Stock bottle to store diluted Wash Solution; 0.2 to 1L.
4. Distilled or deionized water to dilute reagent concentrates.
5. Microwell plate reader at 450 nm wavelength 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.

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)

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 ambient temperature 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): Peroxidase conjugated anti-human IgG in buffer with detergents and antimicrobial as stabilizers. 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 the Anti-ZIKV NS1 Positive Control; net OD>0.5.

·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).

·Run a range of sample dilutions for expected higher positives that allows calcuLation of antibody Titer (when specific titer is at least 4-fold higher than non-immune). See Method C.

·Run samples in duplicate if used for quantitation; non-immunes that are significantly lower than immunes may be run in singlicate. The Calibrators that are used for quantitation, e.g., for between-assay normalization, should be run in duplicate.

When determining titer from a dilution curve, singlicates can be run if more than two dilution points are used for titer calculations.

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 8 Calibrator 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.

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

o Add 100uL of calibrators, samples and controls each to predetermined wells.

o Tap the plate gently to mix reagents and incubate for 60 minutes.

o 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]

o Add 100uL of diluted Anti-Human IgG HRP to each well.

o Incubate for 30 minutes.

o Wash wells 5 times as in step 2.

3. Substrate Incubation [100uL – 15 min]

o Add 100uL TMB Substrate to each well. The liquid in the wells will begin to turn blue.

o 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]

o Add 100uL of Stop Solution to each well.

o Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

o 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.

o 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

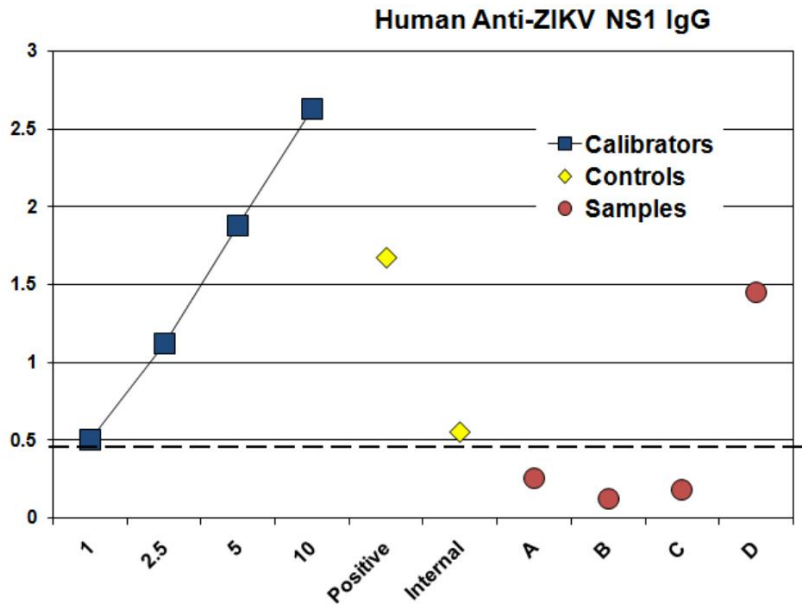
1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (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-NS1 IgG, from either natural infection or vaccination, 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-ZIKV NS1 antibody, derived from NS1 immunization, shows the OD range of the assay; high value indicats optimal sensitivity of the assay.

1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between Positive/Negative. The is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – antiserum reactive to ZIKV NS1; net OD> 0.5. This Control can be used to normalize between-assay variation.

Internal Control – a true positive from an immune human that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). 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 (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 (see Method B):

·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 are Negative for antibody.

Method B. Positive Index

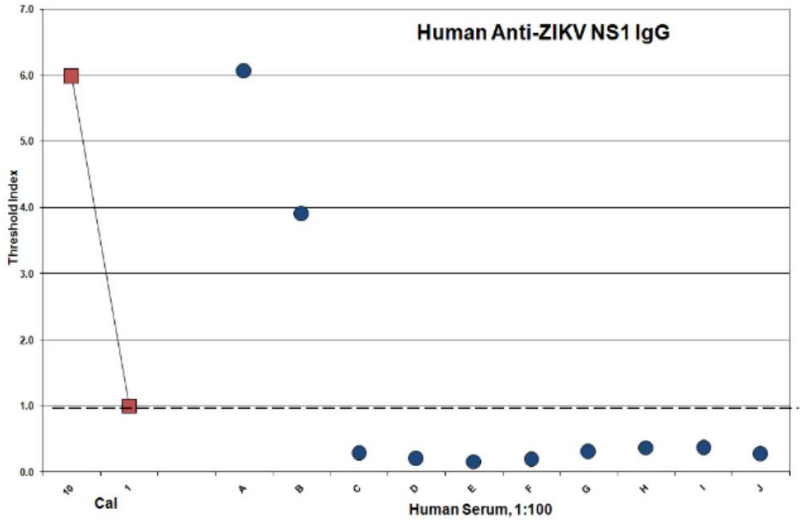
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a Positive Index. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Nonimmune 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 are Negative for antibody.

Example:

Human Serum IgG

A panel of non-immunized human sera was tested for anti-ZIKV NS1 IgG (1:100 dilution). Threshold Index was calculated using the 1 U/ml Cal.



Results

Anti-ZIKV NS1 IgG: two (2) serum pools (A,B) were positive (above the 1.0 threshold); the remaining individual samples were negative (below 1.0 threshold) at 1:100 dilution.

Notes:

1. Positives may be due to prior encounter with the virus or non-Zika proteins with common epitopes, or may be an aspect of the innate immune repertoire.
2. When the Positive Index is above 5.0, using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
3. The sensitivity of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1/500) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1/50) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a Positive Index (see above) or use an Internal Control (Method A).

Method C. Titers from Sample Dilution Curves

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. The titer of elevated 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 Positive and Sensitivity Control values can be used to normalize inter-assay values.

Specificity

Zika NS1 Protein

Zika NS1 protein was expressed as His-tag fusion protein and purified (Uganda MR 766, fuLl length,>95% pure). The NS1 from

African, Asian, and Brazilian Zika strains have 96-100% protein conservation. NS1 proteins from other flaviviruses NS1 show

significant sequence conservation: Spondweni virus (74%), Dengue virus 1/3 (56%), Usutu virus (56%), and Japanese encephalitis virus (57%). Anti-NS1 antibodies from the related flaviviruses may react with the Zika NS1 protein.

Antibody Specificity

The anti-human IgG-HRP used in the kit is specific for human IgG with no significant detection of IgM, IgA, IgE or other antibodies. This kit is optimized for human samples.

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.