INTENDED USE

The **Mouse Anti-Zika Virus Precursor Membrane Protein (ZIKV-prM) IgG2a** ELISA Kit is an immunoassay suitable for quantifying IgG2a antibody activity specific for Zika Virus prM in serum or plasma of vaccinated, immunized, and/or infected hosts.

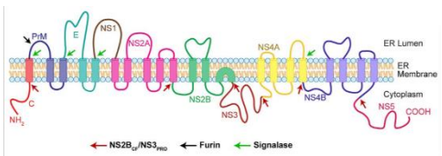
This immunoassay is suitable for:

- 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 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 INFORMATION

Zika virus (ZIKV), a member of the virus family Flaviviridae (flavus means yellow), is 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-borne encephalitis have been developed 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.

PRINCIPLE OF THE TEST

The Anti-ZIKV IgG2a ELISA kits are based on the binding of antibodies (IgG2a) in samples to the recombinant, purified ZIKV antigen immobilized on the microwells. Bound antibody is detected by anti-mouse IgG2a-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 IgG2a 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 (IgG2a) in samples is determined relative to anti-ZIKV Calibrators.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8ºC 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 4ºC for long term and ambient temperature 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 2-8ºC until the kit lot expires or is used up.
Anti-Mouse IgG2a- HRP Conjugate Concentrate (100x) Part: H-MsG2a.2a11, 0.15ml	Peroxidase conjugated anti-mouse IgG2a 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 2-8ºC storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
ZIKV prM Coated Strip Plate	403201	8-well strips (12)	Coated with purified recombinant ZIKV prM, and post-coated with stabilizers.
Anti-ZIKV-prM Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	403202B 403202C 403202D 403202E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-ZIKV prM; in buffer with antimicrobial as stabilizers.
Anti-ZIKV-prM Positive Control	403202-PC	0.65 ml	Antiserum with anti-ZIKV prM reactivity; [Value range on label]
Low NSB Sample Diluent (LNSD)	TBTm Reduces non-specific binding	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 for diluting samples and Anti-Mouse IgG2a HRP Concentrate.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength 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.

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 (p. 5-7) 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 mouse serum with normal levels of IgG and IgM.
- Run the Anti-ZIKV prM Positive Control; value range on label.
- 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).
- 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.

PLATE SET-UP

Bring all reagents to room temperature (18-30ºC) 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 1-5 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 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-Mouse IgG2a HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

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.

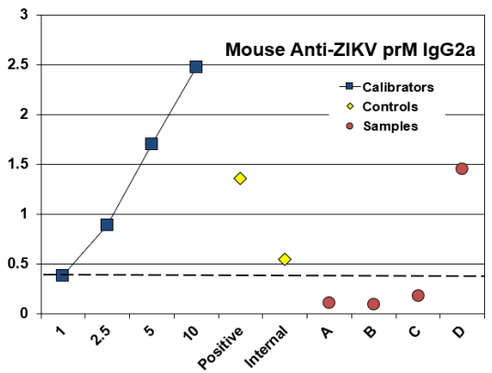
INTERPRETATION OF RESULTS

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-ZIKV prM IgG2a, from either natural exposure or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in mouse 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 prM antibody, derived from prM immunization, 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 high background negatives.

Positive Control – antiserum reactive to ZIKV prM. This Control can be used to normalize between-assay variation.

Internal Control – a true positive from an immune mouse 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.

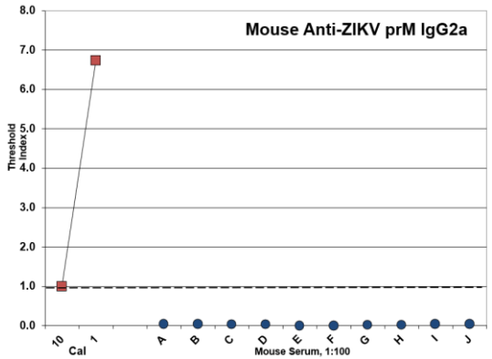
The **1 U/ml** Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative (see p6):

- 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.

ASSAY PERFORMANCE

Example:

Mouse Serum/Plasma IgG2a
A panel of sera/plasma from non-immunized laboratory mice was tested for anti-ZIKV prM IgG2a at 1:100 dilution. **Threshold Index** was calculated using the **1 U/ml Cal**.



Results

Mouse Anti-ZIKV prM IgG2a: all sera (10) 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 below) or use an **Internal Control** (Page 5).

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 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

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 sera dilution.

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

Method C. Titers from Sample Dilution Curves

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.

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

= IgG2a Antibody Activity Units

PRODUCT SPECIFICATIONS

Zika Precursor Membrane Protein

Highly purified Zika precursor membrane protein (Uganda MR 766, full length, >95%, ~10Kda, no tag) is used as coated antigen in this kit. The prM from Zika virus African strain and Brazilian strains are 99% conserved. Zika virus prM sequence shares 61% similarity with Spondweni virus and 50-56% with dengue virus. Flavivirus exposure may produce Zika cross-reactive antibodies.

Antibody Specificity

The anti-mouse IgG2a-HRP used in the kit is specific for mouse IgG2a with no significant detection of IgM, IgA, IgE, or other IgG subclass antibodies. This kit is optimized for mouse samples. ADI has similar kits for human, monkey, and other species.

Instruction Manual No. M-RV-403220-MG2A

Recombivirus™ Mouse Anti-Zika Virus Precursor Membrane (prM) Protein IgG2a ELISA Kit

Cat. #RV-403220-MG2A, 96 tests

For the Detection and Quantitation of Anti-Zika prM IgG2a in Serum or Plasma

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

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ELISA Kit Components	Amount	Part
ZIKV-prM Coated Strip Plate	8-well strip (12)	403201
Anti- ZIKV-prM Positive Control	0.65 ml	403202PC
Anti- ZIKV-prM Calibrator 1 U/ml	0.65 ml	403202B
Anti- ZIKV-prM Calibrator 2.5 U/ml	0.65 ml	403202C
Anti- ZIKV-prM Calibrator 5 U/ml	0.65 ml	403202D
Anti- ZIKV-prM Calibrator 10 U/ml	0.65 ml	403202E
Anti-Mouse IgG2a HRP Conjugate (100X)	0.15 ml	H-MsG2a.2a11
Sample Diluent (20x)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1	RV-403220-MG2A