Package Insert

Zika Virus IgM ELISA

Catalog Number: ZIK31-K01

For Research Use Only. Not for use in diagnostic procedures.

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EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

INTENDED USE

This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the qualitative detection of Zika virus lgM antibodies in blood sample. The assay is useful as an aid in determining recent infection by Zika virus.
For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

ASSAY PRINCIPLE

This "sandwich" ELISA is designed, developed and produced for the qualitative measurement of lgM antibodies in blood specimen The assay utilizes the microplate-based enzyme immunoassay technique by coating highly purified antigen onto the wall of microtiter wells.

Controls and blood specimen are added to microtiter wells of microplate that is coated with highly specific anti human lgM antibodies on its wall. During the assay, Zika antibodies will be bound to the antibody coated plate after an incubation period The unbound material is washed away and biotinylated Zika antigen is added for further immunoreactions. The unbound antigen is washed away and a highly specific monoclonal antibody which is labeled with horseradish peroxidase is added to the wells. After an incubation period, the immunocomplex of "anti human lgM - Zika virus - HRP-conjugated anti Zika virus Tracer Antibody" is formed if Zika lgM antibodies are present in the test sample. The unbound tracer antibody and other proteins in buffer matrix are removed in the subsequent washing step. HRP conjugated tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to Zika lgM antibodies captured on the wall of each microtiter well is directly proportional to the amount of Zika lgG antibody level in each test specimen.

MATERIALS PROVIDED

1. **Anti-human lgM Microtiter Plate**

   One microplate with 12 by 8 strips (96 wells total) coated with Anti-human lgM Specific antibody. The plate is framed and sealed in a foil zipper bag with desiccant. This reagent should be stored at 2 - 8 'C and is stable until the expiration date on the kit box.

2. **Zika virus lgM Negative**

   One vial containing **1 ml** of a normal serum-based control. This reagent is ready to use. It should be stored at 2-B'C and is stable until the expiration date on the kit box.

3. **Zika virus lgM Positive Control**

   One vial containing **0.5 ml** of an anti-Zika virus lgM positive control. This reagent is ready to use. It should be stored at 2- B'C and is stable until the expiration date on the kit box.

4. **Biotin NS-1 Antigen**

   One bottle containing **11 ml** of biotinylated Zika NS-1 virus antigen. This reagent is ready to use. It should be stored at 2 -°8 C and is stable until the expiration date on the kit box.

5. **HRP Conjugated Streptavidin**
   One bottle containing **11 ml** of HRP-conjugated Streptavidin This reagent is ready to use. It should be stored at 2-8'C and is stable until the expiration date on the kit box.

6. **ELISA Wash Concentrate**

   One bottle containing **30 ml** of **30x** wash concentrate. Before use, the contents must be diluted with **870 ml** of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

7. **ELISA HRP Substrate**

   One bottle contains **12 ml** of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent is ready to use and should be stored at 2 - 8'C. It is stable until the expiration date on the kit box.

8. **ELISA Stop Solution**

   One bottle contains **12 ml** of stop solution. It is ready to use. This reagent may be stored at 2 - 8'C or room temperature and is stable until the expiration date on the kit box.

9. **Assay Buffer**

   One bottle contains **15 ml** of assay buffer. This reagent is ready to use. It should be stored at 2-B'C and is stable until the expiration date on the kit box.

SAFETY PRECAUTIONS

The reagents must be used by a iaboratory professional. Reagents of bovine serum were derived in the contiguous 48 United States, and have been obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases.

Wear gloves while performing this assay. Some components of this kit contain human serum. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious.

Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. Upon contact, flush with copious amounts of water for at least 15 minutes. Use good laboratory practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 50 µI, 65 µL, 100 µL, and 1000 µL. 96-well plate or manual strip washer.
2. Repeating dispenser suitable for delivering 100 µL.Paper towels or absorbent paper.
3. Disposable pipette tips suitable for above volume dispensing
4. Incubator or other closed environment at 37°C
5. Disposable plastic 1000 ml bottle with cap.
6. Aluminum Foil
7. Plastic microtitier well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

Specimen Collection

Only 10 µL of human serum or plasma (EDTA) is required for this kit. No special preparation of individual is necessary prior to specimen collection. Blood should be collected by approved medical techniques. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive. particulate should be clarified by centrifugation prior to use. Covered specimens may be stored for up to 48 hours at 2 -8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C and mixed prior to testing. Avoid repeated freeze thaw. At least, two wells of negative and one positive control should be run in every assay.

REAGENT PREPARATION

1. Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate must be diluted to working solution prior to use. Please see REAGENTS section for details.

SAMPLE PREPARATION

Patient serum or plasma sample needs to be diluted 1: 100 with working Sample Diluent (1 x) before being measured.

1. Label test tube (12x75 mm)
2. Add 1mL of assay buffer to each tube
3. Pipet 10 µL of patient serum or plasma sample to tube and mix well

ASSAY PROCEDURE

1. Place a sufficient number of microplate strips in a holder to run assay controls and desired unknown samples in duplicate. See test configuration for example.
2. Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	Negative Ctrl	SAMPLE 3	SAMPLE 7
B	Negative Ctrl	SAMPLE 3	SAMPLE 7
C	Positive Ctrl	SAMPLE 4	SAMPLE 8
D	Positive Ctrl	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	SAMPLE 9
F	SAMPLE 1	SAMPLE 5	SAMPLE 9
G	SAMPLE 2	SAMPLE 6	SAMPLE10
H	SAMPLE 2	SAMPLE 6	SAMPLE 10

3. Dispense **100 µl** of Negative Control, Positive Control and 1: 100 diluted patient serum samples into respective wells.
4. Carefully mix the plate by tapping side gently for 5-10 seconds, making sure not to splash.
5. Cover the plate with aluminum foil to avoid exposure to light. Incubate the plate at **37°C** for **30 minutes.**
6. Wash each well 5 times by dispensing **350 µl** of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
7. Add **100 µl** biotinylaled Zika NS-1 virus Antigen into each well.
8. Cover the plate with an aluminum foil to avoid exposure to light. Incubate the plate at **ROOM TEMPERATURE** for **30 minutes.**
9. Wash each well 5 times by dispensing **350 µl** of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
10. Add **100 µl** HRP Conjugated Streptavidin into each well.
11. Cover the plate with an aluminum foil to avoid exposure to light. Incubate the plate at **ROOM TEMPERATURE** for **30 minutes.**
12. Wash each well 5 times by dispensing **350 µl** of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
13. Add **100 µl** of ELISA HRP Substrate into each well.
14. Cover the plate with an aluminum foil to avoid exposure to light. Incubate plate at room temperature for **20 minutes**
15. Add **100 µl** of ELISA Stop Solution into each of the wells. Mix gently by tapping, making sure not to splash.
16. Read the absorbance at **450 nm** immediately using a microplate reader.

PROCEDURAL NOTES

1. Allow all reagents, especially the microplate wells, to come to room temperature before use. Condensation may form on cold microplate wells when removed from their pouch, which may affect assay results and shelf life.
2. Store any unused antibody coated strips in the foil zip-seal pouch with desiccant to protect from moisture. Exposure of the plates to humidity drastically reduces the shelf life.
3. It is recommended that the negative control and all unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
4. Keep light sensitive reagents in original amber bottles.
5. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
6. Incubation times or temperatures other than those stated in this insert may affect the results.
7. Avoid air bubbles in the microplate wells as this may result in lower binding efficiency and higher CV% of duplicate readings.
8. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

INTERPRETATION OF RESULTS

1. **Validity of the Assay**
    - The negative control should be less than 0.5 at OD 450nm
    - The positive control should be over 0.8 at OD 450 nm
    - If the assay does not meet the above two requirements, the assay is invalid.
2. **Calculation of Cut Off Value (COV)**

    Positive COV = 1.1 x (NCx + 0.10)

    Negative COV = 0.9 x (NCx + 0.10)

    NCX: mean OD of the negative controls
3. **Report Test Sample Result**

	Sample OD
Positive	≥Positive COV
Negative	≤Negative COV
Equivocal	> Negative COV and < Positive COV

Note: For equivocal results, follow up test with a fresh specimen is recommended if clinically indicated

EXAMPLE DATA

ROW	STRIP 1	OD at 450nm
A	Negative Ctrl	0.416
B	Negative Ctrl	0.469
C	Positive Ctrl	1.225

D	Positive Ctrl	1.514
E	Sample	0.505
F	Sample	0.188
G	Sample	0.749

1. The OD of negative controls and positive controls meet the Internal Quality Standard. The Assay is valid.

2. Calculate the Mean OD for negative control:

   $Mean_{neg.} = (0.443 + 0.10) = 0.443$

3. Calculate the Positive and Negative Cut-Off Value:

   Positive COV = 1.1 x (0.443 + 0.10) = 0.597

   Negative COV = 0.9 x (0.443 + 0.10 = 0.489

4. Interpret the Sample Results

   Sample 1 = 0.443 < 0.505 < 0.597 → Equivocal

   Sample 2 = $0.188 \leq 0.489$→ Negative

   Sample 3 = $0.749 \geq 0.597$ → Positive

LIMITATION OF THE PROCEDURE

1. This Zika virus lgM ELISA is limited to the detection of human lgM subtype of antibody against Zika virus in serum or plasma.
2. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. Aspirate the well or get rid of the entire content of wells completely before adding the washing solution.
3. A negative result does not exclude the possibility of exposure or infection with Zika virus.
4. A negative result in neonates must be interpreted with caution, since lgM is not transferred passively in most cases from mother to the fetus before birth.
5. As with all laboratory tests, a definitive clinical decision should not be made based only on the results of a single test. A complete evaluation by physician is needed.
6. Samples with positive or equivocal result must be re-analyzed in duplicate. If both retest values are lower than the cut-off, the final interpretation of the test is negative for Zika virus lgM antibody. If the result is repeatedly positive or equivocal, the sample should be further investigated with other methods.
7. Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.
8. Do not mix or use reagents from different tests that will cause incorrect results.
9. Follow the procedure instructions closely, especially the incubation time and temperature.

QUALITY CONTROL

To ensure the validity of the results each assay must include both negative and positive controls. For a valid test, the positive control must have an absorbance of at least 0.8 OD and the negative control must be less than 0.5 OD. We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

PERFORMACNE CHARACTERISTICS

Reproducibility and Precision

The reproducibility of this assay is validated by measuring two samples both in a single assay of 12-replicate determinations (intra-assay) and in 9 different assays run on different dates (inter-assay)

Inter-Assay Precision			
	Sample 1	**Sample 2**	**Sample 3**
Mean	0.06	0.36	0.13
Std Dev	0.002598	0.12221	0.001641
%CV	4%	3%	1%

Intra-Assay		
	Sample 1	Sample 2
Mean	0.457	0.324
Std Dev	0.018	0.029
%CV	3.0%	9.0

Interference

One positive and one negative sample is added with 5% volume of interference materials to reach a final concentration shown in the table below. All samples are tested in an assay in duplicate.

Sample	Concentration	Interferent added (mg/mL)
Test Control	0.057	
Billirubin	0.056	0.4
	0.054	2
	0.053	10
Test Control	0.058	
Hemoglobin	0.061	0.4
	0.072	2
	0.068	10
Lipid	0.055	8
	0.061	40
	0.055	200

Sample	Concentration	Interferent added (mg/mL)
Test Control	0.820	-
Billirubin	0.821	0.4
	0.833	2
	0.579	10
Test Control	0.827	-
Hemoglobin	0.850	0.4
	0.867	2
	0.871	10
Lipid	0.795	8
	0.726	40
	0.530	200

REFERENCES

1. Center for Disease Control and Prevention
2. Zika Virus, Lyle R. Petersen, M.D , M P .H , Denises J. Jamieson, MD, M.P.H, Ann M. Powers, PH.d , and Margaret A. Honein, Ph D , M.P.H , N Engl J Med 2016; 374:1552-1563 April 2016
3. Zika Virus Infection in Pregnant Women in Rio de Janeiro. Patricia Brasil, MD, Ph.D, Jose P Pereira, Jr , MD , M. Elisabeth Moreira, MD, Ph D, Rita M. Ribeiro Nogueria, MD, Ph D, Luana Damasceno, Pharm D, Mayumi Wakimoto, MD, Ph D et.al., The New England Journal of Medicine

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.