



Dengue virus NS1 Antigen ELISA Kit

Catalog Number KA3214

96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

Dengue virus NS1 Antigen ELISA Kit for early detection of Dengue virus (DENV), is an ELISA assay system for the detection of NS1 antigen in human serum. This test will aid in the early diagnosis of Dengue virus in human serum even prior to the presence of IgM or IgG antibodies. It is not intended to screen blood or blood components, and is for investigational use only. Not for use in diagnostic procedures.

Background

Dengue is an acute viral disease of man, which is transmitted by *Aedes aegypti* mosquitoes. Dengue is characterized clinically by biphasic fever, rash and hematopoietic depression, and by constitutional symptoms such as malaise, arthralgia, myalgia and headache (1). Infrequently, more severe disease is seen, manifested by hemorrhage fever which may progress to lethal shock (2, 3). It is endemic in the tropics and subtropics, worldwide, where an estimated 100,000,000 cases occur annually (4). It has been estimated that about 50 to 100 million cases of Dengue Fever (DF) occur every year with about 250,000 to 500,000 cases of Dengue Hemorrhagic Fever (DHF). During 2002, more than 30 Latin American countries reported over 10,000,000 (DF) cases with large number of DHF cases. This has been followed by extensive epidemics of DHF in several parts of India during 2003 through 2005. In the Americas, the reported incidence has more than tripled from 1996 to 2002. The incidence of Dengue outbreak has been reported in Hawaii (5), and in Laredo, Texas. The potential for the virus to cause a severe disease has also resulted in the inclusion of this pathogen on the CDC "category A" list for potential biological warfare and bioterrorism agents. Dengue NS1 (non-structural) protein is a hexameric secreted protein. It is believed to play a role in viral RNA replication. It is strongly immunogenic eliciting antibodies with complement fixing activity. NS1 antigen can be detected in circulating blood during acute Dengue infection. The Dengue virus NS1 Antigen ELISA Kit can detect NS1 antigen in serum samples almost immediately following infection.

Principle of the Assay

The Dengue virus NS1 Antigen ELISA is a highly sensitive, rapid and reliable assay. It uses one enzymatically amplified "two-step" sandwich-type immunoassay to detect low levels of NS1 in serum.

In this assay, controls and unknown serum samples are diluted in sample dilution buffer, containing secondary antibody, and incubated in microtitration wells. These wells have been coated with a highly effective NS1 antibody and then blocked. NS1 antigens present in the samples are then, "sandwiched" between the capture and secondary antibodies. The presence of NS1 antigen is confirmed by the colorimetric response obtained using an enzyme-conjugate-HRP and liquid TMB substrate. Once the reaction is stopped, using an acidic

solution, the enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. The values obtained for the negative and positive sera serve as guidelines as to determining if a sample contains NS1 antigen.

Note: A set of negative, positive and cut-off controls are provided as internal controls in order to monitor the integrity of the kit components.

General Information

Materials Supplied

List of component

Component	Amount
Coated Microtiter Strips for Dengue NS1: ELISA Strip holder in ziplock foil, containing 96 polystyrene microtiter wells. Store at 2-8°C until expiry.	96 (12x8) well
Dengue NS1 Negative Control: The negative control will aid in verifying the validity of the kit. Store at 2-8°C until expiry. Centrifuge briefly prior to use to sediment any precipitate.	300 µL
Dengue NS1 Positive Control: The positive control will aid in verifying the validity of the kit. Store at 2-8°C until expiry. Centrifuge briefly prior to use to sediment any precipitate	300 µL
Dengue NS1 Cut-Off Control: The cut-off control will aid in determining the cut-off value for the ELISA. Store at 2-8°C until expiry. Centrifuge briefly prior to use to sediment any precipitate.	300 µL
Sample Diluent for Dengue NS1: This solution contains the secondary antibody. Proclin (0.02-0.03%) is added as a preservative. Store at 2-8°C until expiry.	15 mL
100x Conjugate for Dengue NS1: This contains horse radish peroxidase-labeled polyclonal antibody. Mix well prior to use. Store at 2-8°C until expiry.	150 µL
Conjugate Diluent for Dengue NS1: This contains the diluent solution for the 100x Conjugate. The 100x conjugate is diluted directly into this solution. After diluting 100x Conjugate into this solution, the now ready-to-use conjugate may be stored for 2 weeks at 2-8°C before it should be discarded. Store at 2-8°C until expiry.	12 mL
10 X Wash Buffer: Wash Buffer to be used as directed in Test Procedure. Store at 2-8°C until expiry.	120 mL
Liquid TMB Substrate: To be used as directed in Test Procedure. Store at 2-8°C until expiry. <i>Note: This substrate is light-sensitive and should be stored in the original bottle.</i>	12 mL
Stop Solution: To be used to terminate the reaction as directed in Test Procedure. Store at room temperature until expiry. <i>Caution: This is a strong acid, wear protective gloves, mask and safety glasses. Dispose of all materials according to safety rules and regulations.</i>	6 mL

Storage Instruction

Store all reagents between 2-8°C in the dark. Do not remove the adhesive sheets on the unused strips.

Materials Required but Not Supplied

- ✓ ELISA Spectrophotometer capable of absorbance measurement at 450 nm
- ✓ Biological or High-Grade Water
- ✓ Vacuum Pump
- ✓ Automatic Plate Washer
- ✓ 37°C Incubator
- ✓ 1-10 µL Single-Channel Pipettors, 50-200 µL Single- and Multi-Channel Pipettors.
- ✓ Polypropylene tubes or 96 well dilution plates
- ✓ Parafilm
- ✓ Timer
- ✓ Vortex

Precautions for Use

- ✓ Precautions
 - For research or export use only.
 - All human source material used in the preparation of controls has been heat- inactivated. However, all human controls and antigen should still be handled as potentially infectious material. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
 - A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
 - Do not mix various lots of any kit component within an individual assay.
 - Do not use any component beyond the expiration date shown on its label.
 - Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
 - Unused microwells must be resealed immediately and stored in the presence of desiccant. Failure to do this may cause erroneous results.
 - Some reagents may form a slight precipitate, mix gently before use.
 - Incomplete washing will adversely affect the outcome and assay precision.
 - Do not use a humidified chamber for 37°C incubations, as this may affect assay performance.
 - To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the TMB solution.
 - Avoid microbial contamination of reagents, especially of the conjugate concentrate and the conjugate diluent. Avoid contamination of the TMB Substrate Solution with the Enzyme Conjugate-HRP.
 - Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.

- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic materials are being handled.
 - Do not pipette by mouth.
 - Use a clean disposable pipette tip for each reagent, Standard, Control or specimen.
 - Cover working area with disposable absorbent paper.
- ✓ Limitation of procedure:
- Since this is an indirect screening method, the presence of false positive and negative results must be considered.
 - All reactive samples must be evaluated by a confirmatory test.
 - The reagents supplied in this kit are optimized to measure Dengue NS1 levels in serum specimens.
 - Serological cross-reactivity across the flavivirus group is common. Certain sera from patients infected with Japanese Encephalitis, West Nile, and/or Saint Louis viruses may give false positive results. Therefore any Dengue positive sera must be confirmed with other tests.
 - The assay performance characteristics have not been established for visual result determination.
 - Results from immunosuppressed patients must be interpreted with caution.

✓ Potential biohazardous material

This kit may contain reagents made with human serum or plasma. The serum or plasma used has been heat inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

Assay Protocol

Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion

Reagent Preparation

✓ Preparation of 1X Wash Buffer

Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X wash buffer solution, mix 120 ml 10X wash buffer with 1080 ml distilled (or deionized water). Mix thoroughly to ensure that any precipitate is dissolved and that the solution is uniform. Once diluted to 1X, the solution can be stored at room temperature for up to 6 months. Check for contamination prior to use. Discard if contamination is suspected.

✓ Microtitration Wells

Select the number of coated wells required for the assay. The remaining unused wells should be repackaged immediately with the supplied desiccant and stored at 2-8°C until ready to use or expiration.

✓ Preparation of Conjugate Solution

Add 120 µl of 100x Conjugate for Dengue virus NS1 Antigen ELISA directly to the 12 ml bottle of Conjugate Diluent for Dengue NS1 (1 part : 100 parts). Mix by inverting solution several times. This solution may be stored for up to 2 weeks if stored at 2-8°C. After 2 weeks, this conjugate solution should be discarded and no longer used in this assay.

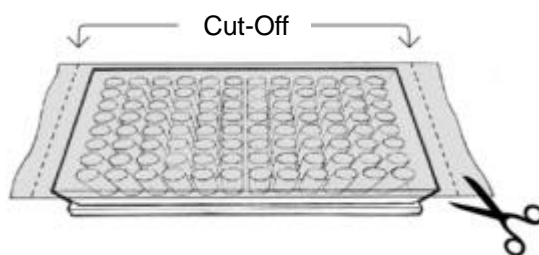
Sample Preparation

1. Human serum must be used with this assay. Reagents have not been optimized, or tested with whole blood or plasma so they cannot be tested directly.
2. Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
3. Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods.
4. Serum should be used and the usual precautions for venipuncture should be observed. The samples may be stored at 2-8°C for up to 7 days, or frozen at -20°C or lower for up to 30 days. To maintain long-term longevity of the serum, store at -70°C. Avoid repeated freezing and thawing of samples.
5. Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.
6. If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.
7. Do not use sera if any indication of growth is observed.

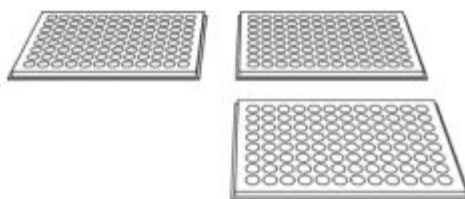
Assay Procedure

1. Positive, negative, and cut-off controls should be assayed in duplicate (and run each time assay is performed on every plate). Unknown serum samples may be tested in singlet. (However, it is recommended to run samples in duplicate until the operator is familiar with the assay.) Ninety test specimens can be tested in singlet on each plate.
2. Using a single channel or multichannel pipettor, aliquot 50 μ l of Sample Diluent for Dengue NS1 ELISA into each of the required wells.
3. Add 50 μ l of each undiluted sera (test samples and control samples) directly to the center of the wells containing the Sample Diluent. Rock the plate gently from side to side 5 times.
4. Cover the top of the plate with parafilm and remove excess.

Note: This is to make sure the temperature distribution is evenly spread out in all wells from bottom and sides; any extra parafilm should be cut-off once the top is sealed to block evaporation.



Note: Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO₂ or other gases. Do not place plates in contact with any wet substances such as wet paper towels etc.



Correct Method

5. Incubate the plate at 37°C for 1 hour in an incubator.

6. After the incubation, wash the plate 6 times with an automatic plate washer using 1x Wash buffer. Use 300 µl per well in each wash cycle.
7. Prepare the Conjugate Solution (120 µl of 100x Conjugate: 12 ml of Conjugate Diluent) and add 100 µl /well of this Conjugate Solution into all wells using a multi-channel pipettor. Discard the remaining Conjugate Solution or store for up to 2 weeks at 2-8°C.
8. Cover the plate with parafilm, as shown above, and incubate at 37°C for 30 minutes in an incubator.
9. After the incubation, wash the plate 6 times with the automatic plate washer using 1x Wash buffer.
10. Add 100 µl per well of Liquid TMB substrate into all wells using a multi-channel pipettor.
11. Incubate the plate in the dark, at room temperature for 20 minutes
12. Add 50 µl per well of Stop Solution into all wells using a multi-channel pipettor and let the plate stand, uncovered at room temperature for 1 minute.
13. Read the optical density at 450 nm (OD₄₅₀) value with a Microplate reader. DO NOT SUBTRACT OR NORMALIZE ANY BLANK VALUES OR WELLS.
14. Record the raw OD₄₅₀ and evaluate the sample status as indicated in the Quality Control section.

✓ Dengue NS1 ELISA Flow Chart

1. Add 50 µl/well of Sample Diluent (containing secondary antibody) for Dengue NS1 ELISA into each of the necessary ELISA plate wells.
2. Add 50 µl of each test sera and control samples to the appropriate wells.
3. Cover and incubate the plate at 37°C for 1 hr, and then wash the plate 6x, 300 µl/well.
4. Prepare the Conjugate Solution (see Reagent Preparation) and apply 100 µl to each ELISA plate well.
5. Cover and incubate the plate at 37°C for 30 minutes, and then wash the plate 6x, 300 µl/well.
6. Add 100 µl/well of Liquid TMB Substrate to the plate.
7. Incubate the plate at RT in a dark place for 20 minutes.
8. Add 50 µl/well of Stop Solution to the plate.
9. Incubate the plate at RT for 1 minute.
10. Read OD₄₅₀ by a plate reader. Do not subtract or normalize any blank values or wells.

Data Analysis

Calculation of Results

✓ Quality Control

Each kit contains positive, negative and cut-off control samples. An acceptable Discrimination Capacity ($R_{PC/NC}$) must be obtained to ensure assay validity. The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cutoff.

The test is invalid and must be repeated if the ($R_{PC/NC}$) value is too low or if the control samples do not meet the specifications. If the test is invalid, the results cannot be used. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to NCCLS C24-A and 42 CFR 493.1256 for guidance on appropriate QC practices. The results below are given strictly for guidance purposes only and applicable for spectrophotometric readings only.

First, calculate the $R_{PC/NC}$ as shown in the example.

- Example:

1. Calculate the mean Negative Control(NC):

Example: Negative Control OD

No 1 0.108

No 2 0.084

Total 0.192

Average of Negative Control = $0.192 \div 2 = 0.096$

2. Calculate the mean Positive Control(PC):

Example: Positive Control OD

No 1 1.112

No 2 1.089

Total 2.201

Average of Positive Control = $2.201 \div 2 = 1.101$

3. Calculate the ratio ($R_{PC/NC}$) between Positive and Negative Control:

Example: ($R_{PC/NC}$) = $1.101 \div 0.096 = 11.47$

Next, ensure that the quality control requirements, listed in the table below, are fulfilled.

- Quality Control Requirements

Control	Requirement
Positive Sample	$OD \geq 0.5$
Negative Sample	$OD < 0.2$
Cut-Off Sample	$OD > \text{Negative Sample}$
$R_{PC/NC}$	≥ 8

- Summary:

The results on the table above must be obtained for the assay to be considered valid. Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

- ✓ Assay Calculations

The status of the unknown sample is determined by first calculating the cut-off of the assay, followed by calculating the ratio of the optical density (OD_{450}) divided by the cut-off.

- Calculation of Cut-off: The cut-off is calculated based on the average OD values obtained with the cut-off control sample.

Example:

Calculate the mean Cut-Off Control:

Example: Cut-Off Control OD

No 1 0.152

No 2 0.189

Total 0.341

Mean of Cut-Off Control = $0.341 \div 2 = 0.171$

Example Cut-Off Value: 0.171

Note: It is recommended to verify cut-off using sera from geographically relevant population.

- ✓ Calculate Immune Status Ratio (ISR): The immune status ratio (ISR) is calculated from the ratio of the optical density (OD) obtained with the test sample divided by the calculated Cut-Off Value. Calculate the ISR for each test sample

Example:

Calculate the ISR for each sample:

Example: Test Sample OD

Test Sample OD 0.431

Test Sample ISR = $OD \text{ of Test Sample} \div \text{Cut-Off Value}$

Test Sample ISR = $0.431 / 0.171 = 2.52$

- Sample Interpretation Chart

Sample Status	ISR
Positive Sample	≥ 1
Negative Sample	< 1

- Calculation of Cut-off: Endemic control sera were not used for the cut-off calculation. It is recommended to verify cut-off using sera from geographically relevant population.
- Interpretation of results: OD values \geq cut-off (ISR values ≥ 1) will be considered positive for the presence of circulating NS1 antigen. Those sera with OD values close to cut-off ($1.1 > \text{ISR} > 0.9$) should be repeated in duplicate to verify sample status.

Resources

References

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Plate Layout

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