Datasheet for ABIN997068

DENV NS1 ELISA Kit

Go to Product page

Overview

Quantity:	96 tests
Target:	DENV NS1
Reactivity:	Dengue Virus
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Dengue NS1 ELISA for early detection of Dengue virus (DENV), is an ELISA assay system for the detection of NS1 antigen in human serum.
Analytical Method:	Qualitative
Detection Method:	Colorimetric
Material not included:	1. ELISA Spectrophotometer capable of absorbance measurement at 450 nm 2. Biological or High-Grade Water 3. Vacuum Pump 4. Automatic Plate Washer

Target Details

Target:	DENV NS1
Abstract:	DENV NS1 Products
Target Type:	Viral Protein
Background:	Dengue is an acute viral disease of man, which is transmitted by Aedes aegypti mosquitoes.

Target Details

Dengue is characterized clinically by biphasic fever, rash and hematopoietic depression, and by constitutional symptoms such as malaise, arthralgia, myalgia and headache. 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. 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, 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 NS1 ELISA can detect NS1 antigen in serum samples almost immediately following infection.

Application Details

Comment:

Quality Control:
Each kit contains positive, negative and cut-off control samples. An acceptable Discrimination Capacity (R) 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) value is too low or if the control samples do not r v PC/NC r 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 as shown in the example. PC/NC Example 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 - 2 = 0.096 Calculate the mean Positive Control(PC): Example: Positive Control OD No 1

Application Details

	1.112 No 2 1.089 Total 2.201 Average of Positive Control = 2.201 - 2 = 1.101 Calculate the ratio (Rpt/nc) between Positive and Negative Control: Example: (R) = 1.101 - 0.096 = 11.47 r v PC/NC. Next, ensure that the quality control requirements, listed in the table below, are fulfilled. Quality Control Requirements 11. 12. 13. 14. 15. 16. 17. Control Requirement Positive Sample OD > 0.5 Negative Sample OD < 0.2 Cut-Off Sample OD > Negative Sample Rpc/nc > 8 ce 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. 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. Limitations of procedure: 1. Since this is an indirect screening method, the presence of false positive and negative results must be considered. 2. All reactive samples must be evaluated by a confirmatory test. 3. The reagents supplied in this kit are optimized to measure Dengue NS1 levels in serum specimens. 4. 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. 5. The assay performance characteristics have not been established for visual result determination. 6. Results from immunosuppressed patients must be interpreted with caution. 7. Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.
Sample Volume:	50 µL
Assay Time:	1 - 2 h
Plate:	Pre-coated

Application Details

Reagent Preparation:	Preparation of Reagents: 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. 7 Preparation of Conjugate Solution Add 120 µL of 100x Conjugate for Dengue NS1 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. Bring all kit reagents and specimens to room temperature before use. Thoroughly mix the reagents and samples before use by gentle inversion.
Assay Procedure:	Bring all kit reagents and specimens to room temperature (approx. 2 °C) before use. Thoroughly mix the reagents and samples before use by gentle inversion.
Calculation of Results:	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 (OD450) 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. 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 - 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. 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 > 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 > ISR > 0.9) should be repeated in duplicate to verify sample status.
Restrictions:	For Research Use only

Handling

Storage:	4 °C
Expiry Date:	12 months