VDx Dengue NS1 ELISA

Microwell ELISA test for detection of Dengue NS1 antigen in Human Serum or Plasma

INTENDED USE:

VDx Dengue NS1 ELISA, is intended to be used for the *in-vitro*, qualitative detection of Dengue NS1 antigens (serotypes DENV1-DENV4), in human serum or plasma.

INTRODUCTION:

Dengue is a mosquito borne tropical infectious disease, caused by the Dengue virus (DENV), which is transmitted primarily through mosquitoes of species Aedes aegypti and to a lesser extent throughAedes albopictus.

There are 4 distinct but antigenically closely related, serotypes of the virus that cause dengue (DENV-1, DENV-2, DENV-3 and DENV-4), the fifth variant DENV-5 has also been isolated in October 2013 in Malaysia, however, there is no indication of the presence of DENV-5 in India.The Dengue virus belongs to the Flaviviridae family of viruses that cause diseases in humans. The infection causes flu-like illness and occasionally develops into a potentially lethal complication such as severe dengue fever.The global incidence of dengueinfection has grown dramatically in recent decades ranging from 50 million to 200 million per year. About half of the world's population is now at risk and more than 125 countries are known to be dengue endemic[2].

The dengue virus RNA, is approximately 11 kb and encodes three structural proteins and seven nonstructural (NS) proteins. NS1 is a highly conserved glycoprotein that seems to be essential for virus viability.

Laboratory diagnosis is essential to confirm dengue and differentiate it from other tropical febrile diseases. The need is for an inexpensive, sensitive and specific assay for the early diagnosis of DENV infection. Thoughthere is no specific treatment for dengue/ severe dengue, however, early detection and access to proper medical care are known to lower fatality rates.

PRINCIPLE OF THE TEST:

I VDx Dengue NS1 ELISA is solid phase Enzyme Linked Immunosorbent Assay, based on the principle ofdirect sand wich immunoassay. In this assay, the polystyrenes microwell strips are coated with highly purified anti-Dengue Ns1 monoclonal antibodies, specific to Dengue NS1 antigens.

ii The sample specimens (serum or plasma),along with positive and negative controls are added in the coated microwells followed by addition of the Enzyme Conjugate (anti- Dengue NS1 monoclonal antibodies linked with horseradish peroxidase (HRPO) and incubated.

iii The wells are washed using the Wash Buffer to remove unbound/nonreactive components .

iv The bound enzyme is detected by addition of the Substrate Buffer and Chromogen-3,3',5',5'-Tetramethyl benzidine (TMB) through the development of a blue color, in case of a sample positive for Dengue NS1 antigen.

v The reaction is stopped by addition of the Stop Solution. A yellow color develops, which is finally read at 450nm using an ELISA reader.

KIT CONTENTS:

i **Microwells ELISA plates :** One plate of 96 microwells coated with anti- Dengue NS1 monoclonal antibodies, packed in sealed aluminum pouch with desiccant. Allow the wells to reach room temperature (18 to 30°C) before removal from the pouch. Place. unused wells in the sealable storage pouch provided and return to 2 to 8°C. Once opened Microwells should be used within one month.

ii **Sample Diluent:** One bottle containing 10 ml of Sample Diluent buffer, containing proteins, stabilizers and preservatives to be used for sample dilution as per the protocol of the test.

iii **Negative Control:** One vial containing 0.3 ml of inactivated and stabilized normal human serum with preservatives. Negative Control has been tested and found to be negative for Dengue NS1 antigen, anti - HIV 1+2 antibodies, HBsAg, Anti - HCV antibodies and Syphilis.

iv **Positive Control:** One vial containing 0.3 ml of inactivated and stabilized human serum with preservatives. Positive Control has been tested and found to be positive for Dengue Ns1 antigen and negative for Dengue NS1 antigen, anti-HIV 1+2 antibodies, HBsAg, Anti - HCV antibodies and Syphilis.

v **Enzyme Conjugate (101x) :** One vial containing 0.3 ml of Enzyme Conjugate (101X) containing anti-Dengue Ns1 monoclonal antibodies linked with horseradish peroxidase (HRPO) with stabilizers. For preparation of the Working Enzyme Conjugate solution, refer to preparation of Working Reagents.

vi **Conjugate Diluent:** One bottle containing 15ml of Conjugate Diluent buffer, containing proteins, stabilizers and preservatives with heterophilic blocking agents, to be used for conjugate dilution.

vii **Substrate Buffer:** One bottle containing 15 ml of Substrate Buffer containing Hydrogen Peroxide(H_2O_2) and Stabilizers. For preparation 0f Working TMB Substrate solution, refer to the preparation of Working Reagents.

viii **TMB Substrate (101x):** One vial containing 0.3 ml of 3,3',5',5'-Tetramethyl benzidine - TMB,(101X) containing Dimethyl sulfoxide (DMSO) as solvent.

ix **Wash Buffer (25X):** One bottle containing 20 ml of oncentrated Wash Buffer (25x) containing phosphate buffer saline with detergents and preservatives.For preparation of Working Wash Solution, refer to preparation of Working Reagents.

x **Stop Solution:** One bottle containing 8 ml of ready to use Stop Solution containing 2N $H_2SO_4$and stabilizers.

xi **Micro strip Cover sealer:** Adhesive sheets to cover microwells strips during incubation.

vii **Product Insert:** Containing detailed information about the kit.

MATERIALS REQUIRED BUT NOT PROVIDED:

(A) Distilled or deionized water, (**B**) Graduated Cylinder for reagents dilutions, (**C**) Micropipettes and Microtips,(**D**) Paper towels or absorbent paper, (**E**) Timer, (**F**) Incubator, maintained at 37±1°C, (**G**) ELISA Reader, (**H**) ELISA Washer, (**I**) Sodium Hypochlorite solution (free available chlorine 50-500mg/dl) and (**J**) Disposable gloves.

STORAGE & STABILITY:

Store the kit and its contents at 2-8°C, upto the expiry date printed on the labels.

SAMPLE SPECIMEN COLLECTION AND HANDLING:

- Only human serum or plasma should be used for the test.
- Specimen should be free of particulate matter and microbial contamination.
- It is preferable to use fresh samples.
- Specimens can be stored at 2-8°C for a short duration of one week.
- For longer storage, freeze the sample specimen at -20 °C or below.
- Avoid repeat freezing and thawing of the samples.
- Do not heat inactivate the sample before use.
- Hemolyzed and hyperlipemic samples may give erroneous results.
- After thawing ,ensure samples are thoroughly mixed before testing.

PRECAUTIONS:

a. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated area swabbed with 1.0% Sodium Hypochlorite solution before work is continued. Sodium Hypochlorite should not be used on acid-containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed off as potentially bio-hazardous waste. Do not autoclave materials containing's Sodium Hypochlorite.

b. Do not pipette by mouth. Wear disposable gloves and eye protection while handlings specimens and performing the assay. Wash hands thoroughly thereafter.

c. Sulphuric acid used in Stop Solution is corrosive and should be handled with appropriate care. If it comes into contact with skin or eyes, wash thoroughly with water.If any of the reagents come in contact with the skin or eyes, wash the area extensively with water.

d. Do not mix the reagents from different batches and do not use the reagents beyond the stated expiry date.

e. Do not modify the test procedure or substitute reagents from other manufactures or other lots.

f. Do not reduce any of the recommended incubation times.

g. Allow the reagents and samples to come to 18 to 30°C before use.Immediately after use, return reagents to the recommended storage temperature.

h. Do not allow wells to become dry during the assay procedure.

I. Do not interchange the caps and cross-contaminate reagents. It is recommended to use dedicated separate pipettes for dispensing the Substrate Solution and Conjugate.

j. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.

PREPARATION FOR THE TEST:

1. Bring all the reagents to room temperature, 30minutes before use.
2. Take out the required number of strips from the sealed ELISA plate pouch.The remaining strips must be kept at 2-8°C with the desiccant after proper sealing of the pouch.

TEST PROCEDURE:

(a) Take the required number of strips and fix them on to the plate.

(b) Pipette 50 µl of Sample Diluent into each micro well.

(c) Add 50µl of Negative Control into B1,C1 & D1 wells and50µl of Positive Control into E1 & F1 wells and then, add 50µl each of the samples to be tested into the remaining wells.

(d) Add 100 µl of Working Conjugate solution in each well. Do not add anything in Blank well A1.

(e) Ensure proper mixing of controls, samples and Working Conjugate for better results.

(f) Incubate at 37±1° C for 90 min. ±1min. after covering the plate with adhesive strip cover sealers provided in the kit.

(g) Take out the plate from the incubator after incubation time is over and then aspirate the contents from each of the wells and wash each well 6 times with 350 µl of Working Wash Solution.

(h) Invert the plate and tap it on absorbent paper to remove the remaining Wash Solution and then, pipette 100 µl of Working Substrate solution into each well.

(I incubate the plate at room temperature (20-30 °C) for 30 minutes, in dark after covering with the adhesive strip cover sealers, provided in the kit. Avoid exposure to light.

(j) Pipette 50 µl of Stop Solution to each well including the Blank well(A1).

(k) Read the absorbance at 450 nm (reference wavelength at 600-650nm) against air within 15 minutes after pipetting the stop solution.

PREPERATION OF WORKING REAGENTS:

A. Preparation of Working Wash solution: Bring the concentrated Wash Buffer (25x) to room temperature and mix well till theappearance becomes clear. If any crystals are observed in the Wash Buffer, keep it at 37°C until the crystals dissolve. Dilute the Wash Buffer with distilled or deionized water to make the Working Wash Solution as below:

Wash Buffer (25X) in ml	1	2	3	4	5	6	7	8
Distilled/DI water in ml	24	48	72	96	120	144	168	192
Total Volume in ml	25	50	75	100	125	150	175	200

The Working Wash Solution is stable at room temperature up to one month, if stored in a clean and closed container.

B. **Preparation of Working Conjugate solution:** Take out the Enzyme Conjugate (101x) from the kit and mix gently. Dilute the Enzyme Conjugate with Conjugate Diluent to make the Working Conjugate solution as below:

No. of Strips	1	2	3	4	6	8	10	12
Conjugate Diluent in ml	1	2	3	4	6	8	10	12
Enzyme conjugate (101x) in µl	10	20	30	40	60	80	101x	120

Prepare the Working Conjugate solution in clean and dried container **just before use** for better performance.

C. Preparation of Working Substrate solution:

No. of Strips	1	2	3	4	6	8	10	12
Substrate Buffer in ml	1	2	3	4	6	8	10	12
TMB Substrate (101x) in µl	10	20	30	40	60	80	101x	120

Prepare the Working Substrate solution in clean and dried container **just before use** for better performance.

QULITY CONTROL:

Results of an assay are considered to be valid, if the following criteria for the Controls are met:

a. Absorbance of Blank should be <0.050.

b. Mean Absorbance of Negative Controls should be < 0.100. If absorbance of the Negative Control reads in the negative, e.g. -0.003 , it should be considered as zero.

c. Mean Absorbance of Positive Controls should be >0.500.

d. If the absorbances obtained do not comply with the ranges mentioned above,the test should be conducted again while following the protocol, meticulously.

INTERPRETATION OF RESULTS:

1. Calculation of the Cut off Value (COV):

Calculate the Negative Control Mean (Ncx) e.g. Negative Control 1 absorbance = 0.009, Negative Control 2 absorbance = 0.010, Negative Control 3 absorbance = 0. 011

- Negative Control Mean (NCx) = (0.009+0.010+0.011)/3=0.010
- Cut off Value (COV) = NCx + 0.200 = 0.010+0.200 = 0.210

Interpretation of Results:

- **Reactive Results: -** Samples with absorbances greater than or equal to the Cut off value are considered Reactive in the assay.
- **Non-Reactive Results: -** Samples with absorbances less than the Cut off value are considered non-reactive in the assay.
- **Gray Zone results:** Samples whose absorbance falls in between ±10% of Cut-off value , should be considered to be in the Gray zone and should be tested within 2-3 days from the date of first bleed.

NOTE:

- If a sample shows a reactive result , the test should be repeated two more times.
- In case the re-tests show non-reactive results, the sample should be considered as non -reactive and if one of the re -tests shows a reactive result, the sample should be considered as reactive.
- The samples considered reactive should be tested again by similar or higher version of the other tests followed by clinical investigation for final interpretation.

LIMITATION OF THE TEST:

- The Dengue NS1-Ag ELISA test should be used for detection of NS1 antigen in serum or plasma only and not in other body fluids.
- This is only a screening test and will only indicate for presence or absence of Dengue NS1antigen in the sample specimen. All reactive samples should be confirmed by a confirmatory test. The definitive diagnosis including patient clinical history, symptoms as well as serological evidence should be considered before reporting final results.

PERFORMANCE CHARACTERISTICS:

No. of true negative samples tested	400	Specificity	99.5%
No. of true positive samples tested	50	Sensitivity	100%

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