

“ELISA Assay for the Detection of Dengue NS1 in Human Serum or Plasma”

Store at 2 °C to 8 °C

INTENDED USE:

The ImmunoELISA Dengue NS1 is a solid phase enzyme linked immunosorbent assay for the qualitative detection of Dengue Non-structural protein (Dengue NS1) in human serum or plasma. It is intended for professional use only as an aid in the identification & diagnosis of infection with Dengue virus. Any reactive specimen with the ImmunoELISA Dengue NS1 Kit must be confirmed with alternative testing method(s) and correlate with clinical findings.

SUMMARY OF TEST:

Dengue (break-bone fever) is a viral infection that spreads from mosquitoes to people. It is more common in tropical and subtropical climates. Most people with dengue have mild or no symptoms and will get better in 1–2 weeks. Rarely, dengue can be severe and lead to death. Most cases of dengue fever can be treated at home with pain medicine. Preventing mosquito bites is the best way to avoid getting dengue. There is no specific treatment for dengue. The focus is on treating pain symptoms. Acetaminophen (paracetamol) is often used to control pain. Non-steroidal anti-inflammatory drugs like ibuprofen and aspirin are avoided as they can increase the risk of bleeding. There is a vaccine called Dengvaxia for people who have had dengue at least once and live in places where the disease is common. For people with severe dengue, hospitalization is often needed.

NS1 tests detect the non-structural protein NS1 of dengue virus. This protein is secreted into the blood during dengue infection. NS1 tests have been developed for use in serum. Most of these tests use synthetically labelled antibodies to detect dengue NS1 protein.

PRINCIPLE OF THE TEST:

ImmunoELISA Dengue NS1 ELISA is a solid-phase enzyme-linked immuno-sorbent assay) based on the "sandwich principle". The solid phase of the microtiter plate is made of polystyrene wells coated with mouse monoclonal antibodies specific for Dengue NS1. Serum or plasma sample is added to the microwells. During incubation, the specific immunocomplex formed in case of presence of Dengue NS1 in the sample is captured on the solid phase. Then the second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) directed against a different epitope of Dengue NS1 is added to the wells.

After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. A color develops in proportion to the amount of antigen bound to Anti-Dengue NS1 mAb coated on the microwells. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength of 630.

KIT COMPONENTS (1 X 96 TEST)

- Microwell plate (1x96 wells):** Each well coated with anti-Dengue NS1 monoclonal antibody in bicarbonate buffer (solvent). The plate is sealed in aluminum pouch with desiccant. The microwell strips can be broken to be used separately.
- Negative Control (1x 2 ml):** One bottle contains normal human serum and Proclin-300, 0.1% v/v as preservative.
- Positive Control (1x 1 ml):** One bottle contains inactivated Dengue NS1 human serum and Proclin-300, 0.1% v/v as preservative.
- Specimen/Sample Diluent (1x 20 ml):** One bottle contains Phosphate buffer saline, Triton-X -100 (as surfactant), Bovine serum albumin (protein stabilizer) and Proclin-300 (as preservative) 0.1% v/v.

- NS1 specific HRP Conjugate concentrate, 51x (1x 0.5 ml):** one bottle contains anti NS1–peroxidase conjugate, Bovine serum albumin (as stabilizer) and gentamycin sulphate 0.005% and Proclin-300, 0.05% v/v (as preservative).
- Conjugate Diluent (1x 20 ml):** one bottle contains phosphate saline-casein buffer and Proclin-300 0.05% v/v (as preservative).
- TMB Substrate, 101x concentrated (1x 0.3 ml):** one bottle contains tetramethyl benzidine in Dimethyl sulphoxide as solvent.
- Substrate Buffer (1x20ml):** one bottle contains Citrate-Acetate buffer (solvent) containing hydrogen peroxide 0.006% v/v.
- Wash Buffer concentrate 20x (1x 50ml):** one bottle contains concentrated phosphate buffered saline with polysorbate (surfactant) and Proclin-300 (preservative), 0.05 %v/v.
- Stop Solution (1x 20ml):** one bottle contains 1.5 N sulphuric acid.
- Instruction manual/product insert.**

MATERIALS REQUIRED BUT NOT PROVIDED:

- Micro pipette capable of delivering 10 µl, 50 µl, 75 µl, and 100 µl volumes with a precision better than 1.5%.
- Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable.
- Absorbent paper for blotting the microplate wells
- Parafilm or other adhesive film sealant for sealing plate
- Timer
- Distilled or de-ionized water.

SPECIMEN COLLECTION AND REPARATION:

- Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum or plasma specimen without additives only.
- If a specimen is not tested immediately, the serum/plasma shall be separated and refrigerated at 2°C - 8°C. If storage period greater than three days are anticipated, the separated serum/plasma should be frozen (-20°C).
- Avoid repeated freezing-thawing of specimens.
- If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
- Do not use serum specimens demonstrating gross lipemic or lipemia, gross hemolysis or turbidity.
- Do not use specimens containing sodium azide as preservative.

REAGENT PREPARATION

- Wash Buffer preparation:** Dilute 20x wash buffer provided in the kit in 1:20 ratio in purified water. For example, to prepare 1000 ml wash buffer, mix 50 ml of 20x wash buffer into 950 ml of purified water. Decide the volume of buffer according to the required number of test and dead volume of washer instrument. In case of manual washing, the volume can be calculated accordingly.

B. HRP Conjugate preparation: Dilute HRP conjugate concentrate (51x) in conjugate Diluent (1:51 ratio) as follows:

| Strips | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------------------|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|
| HRP C. 51x (μl) | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 |
| Conj. Diluent (ml) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

C. TMB substrate preparation: Dilute TMB substrate concentrate (101x) in Substrate Buffer (1:101 ratio) as follows:

| Strips | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----------------------|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| TMB S. 101x (μl) | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 |
| Substrate Buffer (ml) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

- ✓ Prepare the reagents just before use.
- ✓ Prepare only the required quantity of reagents.
- ✓ Ensure the TMB substrate to be kept in low light and closed container.
- ✓ Discard the remaining reagent after use.

ASSAY PROCEDURE

- Take the required number of strips and fix them to plate.
- Pipette 50μl of Negative control into each well from 1A to 1C and 50μl of Positive Control into each well from 1D to 1E, respectively and then, pipette 50 μl of each specimen into the remaining well.
- Add 50μl of Sample diluent into each well.
- Mix the added samples manually or by using microplate shaker, take care not to mix or splash contents out of well while mixing.
- Seal the microwell strips using the plate cover and incubate at 37°C ± 1°C for 60 minutes.
- After completion of 1st incubation, add 100μl of diluted HRP conjugate in each well.
- Mix the added samples manually or by using microplate shaker, take care not to mix or splash contents out of well while mixing.
- Seal the microwell strips using the plate cover and incubate at 37°C ± 1°C for 60 minutes.
- Before 5 to 10 minutes of completion of the 2nd incubation, make 1:101 dilution of TMB substrate with substrate buffer.
- Aspirate the contents from all the wells and wash 6 times with 325μl of diluted washing solution. (325μl/well/time)
- Invert the plate and tap it on absorbent paper to remove the remaining wash solution, and then, pipette 100 μl of prepared diluted substrate into each well. And incubate at controlled room temperature (21°C - 25°C) in dark environment for 30 minutes.
- Pipette 100 μl of stop solution into each well and tap the plate gently to homogenize the coloring materials.
- Read the absorbance at 450 nm (reference wavelength at 630nm) against an air blank within 30 minutes after pipetting of stop solution.

QUALITY CONTROL

- The average absorbance of Positive Control (PCx) should be greater than or equal to 1.0.
- The average absorbance of Negative Control (NCx) should be less than or equal to 0.200.

INTERPRETATION OF RESULTS

(a) Calculate the negative control mean (NCx)

Ex. Negative Control 1 absorbance=0.045
 Negative Control 2 absorbance=0.050
 Negative Control 3 absorbance=0.055
 Negative Control Mean (NCx)=
 $(0.045+0.050+0.055)/3=0.050$

(b) Calculate the positive control mean (PCx)

Ex. Positive Control 1 absorbance=2.13
 Positive Control 2 absorbance=2.05
 Positive Control Mean (PCx)= $(2.13+2.05)/2=2.09$

(c) Calculate the cut off value

Cut off value= NCx + 0.200

INTERPRETATION

Samples with absorbance greater than or equal to the Cut Off value are considered positive to Dengue NS1. Samples with absorbance less than Cut Off value are considered negative to Dengue NS1. Sample values within the ±10% of cut off value should be considered as indeterminate or grey zone samples. It should be retested again or repeat with freshly collected sample.

WARNING AND PRECAUTIONS:

- This product is made for in Vitro Diagnostic Use only.
- Package insert must be read completely before performing the test. Failure to follow the instructions in pack insert may give inaccurate test results.
- Do not use the Kit beyond the expiry date.
- Bring all reagents & samples to room temperature (18°C-28°C) before use.
- Do not use the components in any other type of test kit as a substitute for the components in this kit.
- Do not use hemolyzed blood specimen for testing.
- Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, and other blood-borne pathogens.
- Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
- In the beginning of each incubation and after adding Stop Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as it may give inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells.
- Don't allow the micro plate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate solution.
- The TMB substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate must be stored in the dark.
- Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
- The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.












17. Avoid strong light or sunlight during color development.

LIMITATIONS:

1. The **ImmunoELISA Dengue NS1** is intended to detect antigen against Dengue NS1. However, the test cannot detect the quantity or relative change in the levels of antibodies.
2. The product is not made for detection of Dengue NS1 Antibody.
3. The product is designed so as to give best results but, some samples may show cross reactivity. Hence, there may be a chance of false positive results. Every positive result shall be verified by testing with alternate kit or methods and also shall be correlated with symptoms.

REFERENCES:

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2. Dengue Outbreak Investigation Team. Dengue fever, Hawaii, 2001-2002. Emerg Infect Dis. 2005; 11(5) Alcon-LePoder S, Sivard P, Drouet MT, Talarmin A, Rice C, Flamand M. Secretion of flaviviral non-structural protein NS1: from diagnosis to pathogenesis.
3. Songee L. ranch and Paul N. Levett. Evaluation of four methods for detection of immunoglobulin M antibodies to dengue virus. Clin.Diagn. Lab. Immunol. Vol6 (4) p 555-557,1999.
4. Seth, J. (1991). standardization & quality assurance. In principle and practice of immunoassay, Ed. C.P. Price & D.J. Newman. Macmillan Publishers, pp.154-189.

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|  | In Vitro Diagnostic Use |
|  | Manufacturer |
|  | Manufacturing Date |
|  | Expiry Date |
|  | Lot Number |
|  | Store at + 2°C to + 8°C |
|  | Single Use |
|  | Number of tests in the pack |
|  | Do not use if pouch or kit damaged |
|  | This side Up |
|  | Read package insert before use |



MANUFACTURED BY

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