ImmunoELISA Dengue IgG

"ELISA Assay for the Detection of Dengue IgG Antibodies in Human Serum or Plasma"

Store at 2 °C to 8 °C

INTENDED USE:

The ImmunoELISA Dengue IgG is a solid phase enzyme linked immunosorbent assay for the qualitative detection of Dengue IgG antibodies 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 IgG** 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 IgG ELISA is a solid-phase enzyme-linked immuno-sorbent assay) based on the "MAC Capture ELISA". The solid phase of the microtiter plate is made of polystyrene wells coated with Anti-human IgG monoclonal antibodies. Diluted serum or plasma sample is added to the microwells and incubated along with biotin conjugated Dengue antigen. During incubation, the specific antibody – antigen immunocomplex formed in case of presence of Dengue IgG antibodies in the sample is captured on the solid phase. Then the second antibody conjugated with the enzyme horseradish peroxidase (SA-HRP Conjugate) forms Streptavidin-biotin complex.

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 Dengue IgG antibody bound to Anti-human IgG 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)

1. **Microwell plate (1x96 wells)**: Each well coated with anti-human IgG 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.
2. **Negative Control (1x 2.5 ml):** One bottle contains normal human serum and Proclin-300, 0.1% v/v as preservative.
3. **Positive Control (1x 2.5 ml):** One bottle contains inactivated Dengue IgG human serum and Proclin-300, 0.1% v/v as preservative.
4. **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.
5. **Biotin Conjugate (1x 10 ml):** One bottle contains Phosphate buffer saline, biotinylated dengue recombinant antigen (serotype 1-4), Triton-X -100 (as surfactant), Bovine serum albumin (protein stabilizer) and Proclin-300 (as preservative) 0.1% v/v.
6. **Streptavidin HRP Conjugate concentrate, 51x (1x 0.5 ml)**: one bottle contains anti-SA–peroxidase conjugate, Bovine serum albumin (as stabilizer) and gentamycin sulphate 0.005% and Proclin-300, 0.05% v/v (as preservative).
7. **Conjugate Diluent (1x 20 ml)**: one bottle contains phosphate saline-casein buffer and Proclin-300 0.05% v/v (as preservative).
8. **TMB Substrate, 101x concentrated (1x0.3 ml)**: one bottle contains tetramethyl benzidine in Dimethyl sulphoxide as solvent.
9. **Substrate Buffer (1x20ml)**: one bottle contains Citrate-Acetate buffer (solvent) containing hydrogen peroxide 0.006% v/v.
10. **Wash Buffer concentrate 20x (1x50ml)**: one bottle contains concentrated phosphate buffered saline with polysorbate (surfactant) and Proclin-300 (preservative), 0.05 %v/v.
11. **Stop Solution (1x20ml)**: one bottle contains 1.5 N sulphuric acid.
12. **Instruction manual/product Insert.**

MATERIALS REQUIRED BUT NOT PROVIDED:

1. Micro pipette capable of delivering 10 µl, 50 µl, 75 µl, and 100 µl volumes with a precision better than 1.5%.
2. 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.
3. Absorbent paper for blotting the microplate wells
4. Parafilm or other adhesive film sealant for sealing plate
5. Timer
6. Distilled or de-ionized water.

SPECIMEN COLLECTION AND REPARATION:

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

REAGENT PREPARATION

A. **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 TMBsubstrate 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

1. Take the required number of strips and fix them to plate.
2. 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 1:100 diluted specimen into the remaining well.
3. Add 50µl of biotin conjugate into each well.
4. Mix the added samples manually or by using microplate shaker, take care not to mix or splash contents out of well while mixing.
5. 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 1st incubation, make 1:51x dilution of HRP conjugate.
6. After completion of 1st incubation, aspirate the contents from all the wells and wash 5 times with 325µl of diluted washing solution. (325µl/well/time)
7. Invert the plate and tap it on absorbent paper to remove the remaining wash solution, and then, pipette 100 µl of prepared diluted HRP conjugate into each well.
8. Mix the added samples manually or by using microplate shaker, take care not to mix or splash contents out of well while mixing.
9. Seal the microwell strips using the plate cover and incubate at 37°C ± 1°C for 30 minutes.
10. Before 5 to 10 minutes of completion of the 2ndincubation, make 1:101 dilution of TMB substrate with substrate buffer.
11. After completion of 2nd incubation, aspirate the contents from all the wells and wash 5 times with 325µl of diluted washing solution. (325µl/well/time)
12. Invert the plate and tap it on absorbent paper to remove the remaining wash solution, and then, pipette 100 µl of prepared diluted TMB substrate into each well and incubate at controlled room temperature (21°C - 25°C) in dark environment for 15 minutes.
13. Pipette 100 µl of stop solution into each well and tap the plate gently to homogenize the coloring materials.
14. Read the absorbance at 450 nm (reference wavelength at 630nm) against an air blank within 30 minutes after pipetting of stop solution.

QUALITY CONTROL

1. The average absorbance of Positive Control (PCx) should be greater than or equal to 1.0.
2. 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 IgG antibodies. Samples with absorbance less than Cut Off value are considered negative to Dengue IgG antiodies. 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:

1. This product is made for in Vitro Diagnostic Use only.
2. Package insert must be read completely before performing the test. Failure to follow the instructions in pack insert may give inaccurate test results.
3. Do not use the Kit beyond the expiry date.
4. Bring all reagents & samples to room temperature (18°C-28°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimen for testing.
7. 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.
8. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
9. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV,and other blood-borne pathogens.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. 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.

12. Don't allow the micro plate to dry between the end of the washing operation and the reagent distribution.
13. 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.
14. 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.
15. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
16. The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquidreagents. Insufficientwashing will result in poor precision and falsely elevated absorbance.
17. Avoid strong light or sunlight during color development.

LIMITATIONS:

1. The **ImmunoELISA Dengue IgG** is intended to detect antibodies against Dengue antigen. 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 antigen.
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.

	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 beforeuse

MANUFACTURED BY

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