MaxLISA Dengue NS1 Antigen ELISA

1. Intended Use:
MaxLISA Dengue NS1 antigen ELISA is an in vitro enzyme immunoassay for the qualitative determination of Dengue NS1 antigen human serum and plasma.

Ordering Information

Ref./Cat. No.	Pack Size	Presentation
AVEL-DNS1-48	48 Wells	ELISA
AVEL-DNS1-96	96 Wells	ELISA

2. Summary and Explanation:
The mosquito-borne dengue viruses (serotype 1-4) cause dengue fever, a severe flu-like illness. The disease is prevalent in third world tropical regions and spreading to sub-tropical developed countries - including the United States. WHO estimates that 50-80 million cases of dengue fever occur worldwide each year, including a potentially deadly form of the disease called dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Primary infection with dengue virus results in a self-limiting disease characterized by mild to high fever lasting 3 to 7 days, severe headache with pain behind the eyes, muscle and joint pain, rash and vomiting. Secondry infection is the more common form of the disease in many parts of Southeast Asia and South America. This form of the disease is more serious and can result in DHF and DSS. The major clinical symptoms can include high fever, haemorrhagic evets, and circulatory failure, and the fatality rate can be as high as 40%. Early diagnosis of DSS is particularly important, as patients may die within 12 to 24 hours.

3. Test Principle:
This ELISA kit is designed, developed, and produced for the qualitative detection of the Dengue NS1 antigen present in serum/plasma . A monoclonal antibody specific for Dengue NS1 has been pre-coated onto a microplate well. Standards and samples are pipetted into the wells, and then a horseradish peroxidase-conjugated detection antibody specific for Dengue NS1 is added to the wells, producing an antibody-antigen-antibody sandwich complex. Following incubation and wash steps a substrate is added. A colored product is formed in proportion to the amount of Dengue NS1 present in the sample. The reaction is terminated by the addition of acid and absorbance is measured at 450/620 nm.

4. Key Contents:
Store all components at 2-8°C when not in use.

Material	96 Tests
Dengue NS1 Coated Plate (1 x 96 well microplate)	Microplate coated with monoclonal antibody specific for Dengue NS1,packed in a pouch with desiccant
Positive Control (1 x 1.0 ml)	Ready to use
Negative Control (1 x1 ml)	Ready to use
NS1 Diluent (1 x 20ml)	Buffer solution containing stabilizing proteins and preservatives and detergent
Conjugate Concentrate (100X) (1 x 0.22 ml)	HRP conjugate to be diluted with conjugate diluent.
Wash Buffer (20X) (1 x 30 ml)	Buffer containing surfactants
TMB Substrate A (1 x 10ml)	Buffer solution containing H_2O_2 with preservative.
TMB SubstrateB (1 x 10ml)	To be diluted in TMB diluent before use.
Stop solution (1 x 15 ml)	Ready to use, 0.1N Sulfuric acid
Pack insert	

5. Materials required but not provided:
- Distilled or deionized water.
- Micropipettes and Micro tips.
- Graduated cylinders for reagent bottles.
- Paper towels or Absorbent tissue.
- 70% Isopropanol solution.
- Vortex mixer.
- Incubator (37°C).
- ELISA Washer.
- ELISA Reader.
- Timer.
- Biohazard waste container with sodium hypochlorite solution.
- Disposable gloves

6. Sample collection, Preparation and Storage:
- Serum/plasma samples can be separated from the blood collected through vein puncture

7. Precautions:
- For in vitro diagnostic use only
- Bring all reagents and specimen to room temperature before use.
- The use of disposable gloves and proper biohazards clothing is
- strongly recommended while running the test.
- Do not eat, drink or smoke in the area where testing is done
- In case there is a cut/wound in hand, do not perform the test.
- Do not pipette any material by mouth.
- Do not mix components of one kit with another.
- Do not allow liquid from one well to mix with other wells.
- Do not let the strips dry in between the steps.
- All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C for 60 min. Do not autoclave materials or solution containing sodium
- hypochlorite. They should be disposed off in accordance with established safety procedures.
- Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
- Stop solution contains sulfuric acid. If sulfuric acid comes in contact with the skin, wash thoroughly with water. In case of contact with eyes, flush with excess of water.

8. Preparation of Reagents:
Note: Before use, allow reagents and samples at room temperature (20- 30°C).
8.1. Ready for use reagents:
8.1.1. Microplate:
Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissors or a scalpel above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at ±2-8°C.
Caution: Handle Microwell strips with care. Do not touch the bottom exterior surface of the walls.
8.1.2. Negative control
8.1.3. Positive control
8.1.5. Sample diluent
8.1.6. Stop solution
8.2. Reagents to reconstitute:
8.2.1. Wash buffer (20X):
- Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals dissolve.
- Dilute 1:20 in distilled water to obtain the ready to use washing solution. Mix 20 ml of 20X wash buffer concentrate with 380ml of distilled or deionized water. Working wash buffer is stable for 15 days when stored at 2-8°C.

8.2.2. Wash Procedure:

- Incomplete washing will adversely affect the test outcome.
- Aspirate the well contents completely into a waste container.
- Then fill the wells completely with wash buffer avoiding overflow of buffer from one well to another well.
- Aspirate completely and repeat the wash procedure for a total 5 times of washes.
- Automated washer if used should be well adjusted to fill each well completely.
- Tap plate on absorbent papers till no droplets appear on the paper

8.2.3. Preparation of working conjugate:

Make a 1:100 dilution of conjugate concentrate with conjugate diluent. Do not store working conjugate. Prepare conjugate 10 minutes before use. Determine the quantity of working conjugate solution to be prepared from table given below. Mix solution thoroughly before use.

Example

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
Conjugate concentrate 100 X (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Conjugate Diluent (ml)	1	2	3	4	5	6	7	8	9	10	11	12

8.2.4. Preparation of Substrate:

Mix equal amount of TMB substrate A and B

9. Test procedure:

Bring all the reagents and specimen to room temperature before use.

1. Take the required number of strips and fix them to frame and immediately close the pouch.
2. Prepare template in data sheet indicating the location of controls and specimens.
3. Add 100µl of negative control A1, B1
4. Add 100µl Positive control in C1 wells respectively.
5. Add 50 µl of Diluent in each well starting from D1.
6. Add 50 µl of Patient sample in each well starting from D1
7. Add 100µl of conjugate solution in each well.
8. Mix gently and cover plate with aluminum foil and incubate for 90 minutes at 37°C.
9. After incubation, aspirate the contents from all the wells and wash each well 6 times with by filling approximately 300µl diluted wash buffer.
10. Invert the plate and tap it on absorbent paper to remove the remaining washing solution, and then pipette 150µl of prepared diluted substrate into each well and incubate at room temperature for 30 minutes in DARK.
11. Add 100µl of stop solution each well.
12. Read absorbance at 450/620nm within 10 minutes in ELISA READER

10. Flow chart for Test Run criteria:

Add 100µl of negative control solution in A1 & B 1 well.

↓

Add 100µl of Positive control solution in C1 well.

↓

Add 50 µl of diluent in each well starting from D1 followed by 50 µl Patient sample

↓

Add 100µl of conjugate solution in each well

↓

Cover plate with aluminum foil and incubate for 90mins at 37°C

↓

Wash each well 6 times with 300µl of wash buffer and dry with absorbent paper

↓

Add 150µl of substrate solution in each well and incubate at RT for 30mins

↓

Add 100µl of stop solution to stop reaction

↓

Read results at 450/620nm within 10 minutes in ELISA READER

11. Calculation of the cut-off value:

Negative control (NCx)

Absorbance of Negative control (NC)	
Negative Control 1	0.022
Negative Control 2	0.020
Negative Control Mean (NCx)	(0.022+0.020)/2 = 0.021

b. Cut off value: NCx+0.200 = 0.021 + 0.200 = 0.221

c. Positive control acceptance criteria:

PC must be ≥ 0.50

NC must be ≤ 0.2

d. Abbreviations:

NC - Absorbance of Negative control

NCx - Mean Negative control

PC - Absorbance of the Positive control

12. Interpretation of Results:

- If OD of the samples is greater than cut off value, sample is positive
- If OD of the sample is less than cut off value samples is negative

Manufactured in India by :

AVECON Healthcare Pvt. Ltd.

Transforming Research into Innovations

Plot No.: # 338, Sector-2, Industrial Growth Centre, Saha (Haryana) India-133104.

E-mail : helpdesk@aveconhealthcare.com, Website : www.aveconhealthcare.com

Customer Care No. : +91 93065 12576