KRISHGEN PUDGALA
PARTNER FOR PRECISION DIAGNOSTICS

GENLISA™ Dengue Antigen NS1 ELISA

REF : KBD770

Ver 1.4

IVD

Enyzme immunoassay for Qualitative Determination of Dengue Antigen NS1 in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBD770 Σ 96 tests

Krishgen Pudgala LLP Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400018. India.
Tel: +91-22-49198700 | email: sales@krishgenpudgala.com

GENLISA™ Dengue Antigen NS1 ELISA

Introduction:

Dengue is an acute febrile illness. The term dengue fever came into general use after 1828. The disease is transmitted by a bite of specific female *Aedes* mosquito. Dengue viruses (DV) belong to family *Flaviviridae* and consist of four antigenically distinct serotypes of the virus referred to as DV-1, DV-2, DV-3 and DV-4. DV is a positive-stranded encapsulated RNA virus and comprises of three structural protein genes (capsid, membrane, envelop) and seven non-structural (NS) proteins. All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self-limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). Primary infection with dengue virus is characterized by elevation in specific IgM antibody levels 3 to 5 days after the onset of symptoms; this generally persists for 30 to 60 days, IgG levels also become elevated after 10 to 14 days and remain detectable for life. During secondary infection, IgM levels generally rise more slowly and reach lower levels than in primary infection, while IgG levels rise rapidly from 1 to 2 days after the onset of symptoms and can result into DHF/DSS.The major clinical symptoms can include high fever, hemorrhagic events, and circulatory failure, and the fatality rate can be as high as 40%. The presence of joints and muscle pain gives it an alternative name as “break bone fever.

Intended Use:

The GENLISA™ Dengue Antigen NS1 ELISA is intended for the qualitative determination of NS1 antigen in human serum and plasma. The GENLISA™ Dengue Antigen NS1 ELISA is highly specific as it uses a mixture of Anti-Dengue virus (DENV-NS1 Serotype 1), Anti-Dengue virus (DENV-NS1 Serotype 2), Anti-Dengue virus (DENV-NS1 Serotype 3), Anti-Dengue virus (DENV-NS1 Serotype 4) as capture antibody in a sandwich format.

Principle:

Dengue Antigen NS1 GENLISA™ ELISA is a sandwich enzyme linked immnunosorbent assay for qualitative determination of NS1 antigen present in the human serum and plasma. Anti-Dengue virus NS1 antibody is pre-coated onto microwells. Samples, Controls are pipetted into microwells and Dengue Antigen NS1 present in sample binds to the antibody coated on the wells. Enzyme conjugate antibody is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the TMB substrate is added to microwells and color develops proportionally to the amount of Dengue Antigen NS1 present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti-Dengue NS1 Antibody Microtiter Coated Plate (12 x 8 wells) - 1 no
2. Negative Control - 2 ml
3. Positive Control - 2 ml
4. Cut-Off Control – 2 ml
5. Anti-Dengue NS1:HRP Conjugate - 6.5 ml
6. (20X) Wash Buffer - 25 ml
7. Sample Diluent - 50 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Preparation before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

For Serum / Plasma - Samples have to be diluted 1:5 (v/v), e.g. 25 ul samples + 100 ul sample diluent prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 25ml of Wash Buffer (20X) to 475ml of DI water. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **100 ul Controls,** diluted **Sample and 50 ul** of **Anti-Dengue NS1:HRP Conjugate** in respective wells.
3. Seal the plate and Incubate at 37°C for 120 minutes.
4. Aspirate and wash plate 4 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5. Add **100 ul** of **TMB Substrate** into each well.
6. Incubate at RT for 30 minutes.
7. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Calculation of Results:

Calculation for the samples is done as a ratio of the Mean Sample (duplicates) OD450nm value (S) and the Cut-Off Control (Co), or S/Co

Interpretation of Results:

Test results are interpreted with the S/Co ratio, according to the following table:

S/Co	Interpretation	
< 0.9	Negative	No antigen present against specific pathogen.
0.9 – 1.0	Equivocal	Antigen against the pathogen could not be detected clearly.
> 1.0	Positive	Antigens against the pathogen are present.

Criteria of Validation:

Dengue NS1 antigen results are considered to be valid, if

Negative Control	O.D < 0.25
Positive Control	O.D >= 0.5

Clinical Validation:

120 known positive Dengue NS1 samples and 5 known Dengue NS1 negative samples were run using GENLISA™ Dengue Antigen NS1 ELISA kit.

All the 120 positive samples showed positive results while the 5 negative samples showed negative results indicating 100% specificity

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in-vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Safety Precautions:

- **This kit is For In-vitro Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.

- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply

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This Limited Warranty states the entire obligation of Krishgen Pudgala LLP with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

THANK YOU FOR USING KRISHGEN PRODUCT!

Krishgen Pudgala LLP

Unit No.1/2, Om Sainath Commercial Complex,
Off Mankoli-Anjur Phata Road. Village Dapode, Bhiwandi 421302.

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **100 ul Controls, diluted Samples and 50 ul of Anti-Dengue NS1:HRP Conjugate** in appropriate wells.
3	Seal the plate and Incubate at 37^0C for 120 minutes.
4	Aspirate and **wash plate 4 times** with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5	Add **100 ul** of **TMB Substrate** into each well.
6	**Incubate** at **RT** for **30 minutes**.
7	Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

SYMBOLS KEY

MTP	Coated Microtiter Plate (12 x 8 wells)
PC	Positive Control
NC	Negative Control
CUTOFF CNTRL	Cut-Off Control
HRP CONJ	Anti-Dengue NS1:HRP Conjugate
SAMP DIL	Sample Diluent
20x WASH BUF	(20x) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature