Dengue virus NS1 ELISA kit

Enzyme Immunoassay kit for the quantification of Dengue virus NS1 in serum, plasma and cell culture supernatant samples.

Catalog number: ARG81357

Package: 96 wells

TABLE OF CONTENTS

SECTION **Page**

INTRODUCTION 3

PRINCIPLE OF THE ASSAY 3

MATERIALS PROVIDED & STORAGE INFORMATION 4

MATERIALS REQUIRED BUT NOT PROVIDED 5

TECHNICAL HINTS AND PRECAUTIONS 5

SAMPLE COLLECTION & STORAGE INFORMATION 6

REAGENT PREPARATION 7

ASSAY PROCEDURE 9

CALCULATION OF RESULTS 10

EXAMPLE OF TYPICAL STANDARD CURVE 11

QUALITY ASSURANCE 11

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan (R.O.C.)

Phone: +886 (3) 621 8100

Fax: +886 (3) 553 0266

Email: info@arigobio.com

INTRODUCTION

Dengue virus NS1 protein is a nonstructural protein which could be secreted and have been developed as diagnostic biomarker for early detection. There are several forms of NS1 including monomer, dimer, and hexamer during infection. Dimeric NS1 can be anchored to cell membranes with glycosyl-phosphatidylinositol (GPI). Hexameric NS1 can be secreted and detected in patients' blood samples (up to 50 µg/mL) or infected cell supernatants (various from ng/mL to µg/mL depend on serotypes and strains). Studies have shown that NS1 could interfere complement activity and prothrombin activation. In addition, NS1 could elicit antibodies which cross-react with host antigens including coagulation factors and molecules expressed in endothelial cells and platelets through molecular mimic.

Dengue virus (DENV) non-structural protein 1 (NS1) is involved in virus replication and regulation of the innate immune response. Soluble and membrane-associated NS1 may activate human complement and induce host vascular leakage. This effect might explain the clinical manifestations of dengue hemorrhagic fever and dengue shock syndrome (By similarity). [Uniprot]

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique for the detection of Dengue virus NS1 in serum, plasma and cell culture supernatant samples. An antibody specific for DENV NS1 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any DENV NS1 present is bound on the plate. After washing away any unbound substances, a Horseradish Peroxidase (HRP) conjugated primary antibody binds

to DENV NS1 is added to each well and incubates. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of total DENV NS1 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of total DENV NS1 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
antibody-coated microplate	12 X 8 strips	4°C
Standard (200 ng)	1 vial	4°C, aliquot & store at -20 °C after reconstitution
Standard reconstitution buffer	1.1 ml (ready-to-use)	4°C
HRP-antibody Conjugate (200X)	65 µl	4°C
Antibody diluent buffer	12 ml (ready-to-use)	4°C
Assay Buffer	12 ml (ready-to-use)	4°C
10X Wash buffer	20 ml	4°C
TMB substrate	12 ml (ready-to-use)	4°C (Protect from light)
STOP solution	6 ml (ready-to-use)	4°C
Plate sealer	1 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 1X PBS
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved. 1X Wash buffer should be prepared and stored at 4°C before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. It is important to ensure a platelet free preparation since platelets can release HMGB1. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants: Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **Standard**: Reconstitute the standard with **1.0 ml** Standard reconstitution buffer to yield a stock concentration of **200 ng/ml**. Vortex the tube for 1 min and stand for 10 minutes to completely dissolve contents. Aliquot and store at ≤ -20°C for long term storage.

  Make sure the standard is dissolved completely before making serial dilutions. The 1X PBS serves as zero standard (0 ng/ml), and the standard stock can be diluted with 1X PBS as according to the suggested concentration below: **100 ng/ml**, **50 ng/ml**, **25 ng/ml**, **12.5 ng/ml**, **6.25 ng/ml**, **3.125 ng/ml** and **1.5625 ng/ml**.

  Dilute DENV NS1 standard as according to the table below:

Standard	DENV NS1 Conc. (ng/ml)	µl of 1X PBS	µl of standard
S7	100	250	250 (200 ng/ml Stock)
S6	50	250	250 (S7)
S5	25	250	250 (S6)
S4	12.5	250	250 (S5)
S3	6.25	250	250 (S4)
S2	3.125	250	250 (S3)
S1	1.5625	250	250 (S2)
S0	0	250	0

- **1X Wash buffer**: Dilute 10X wash buffer with distilled water to yield 1X wash buffer. The diluted Wash buffer should be stored at 4 °C.
- **HRP-antibody Conjugate:** Diluent 200X HRP-antibody Conjugate into 1X Antibody diluent buffer to yield 1X Detection antibody solution. The 1X HRP-antibody Conjugate solution should be used immediately.

- Sample:

  - If the sample volume is less than 100 µl, add 1X PBS to a final volume of 100 µl, for the calculation of the concentrations this dilution factor has to be taken into account.

  - If the initial assay found samples contain DENV NS1 higher than the highest standard, the samples can be diluted with 1X PBS and re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  a) Dilution 1:10: 20 µl sample + 180 µl 1X PBS (mix thoroughly).

  b) Dilution 1:100: 20 µl 1:10 diluted a) + 180 µl 1X PBS (mix thoroughly).

  Note: it is recommended to do pre-test to determine the suitable dilution factor.

ASSAY PROCEDURE

All materials do not need equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

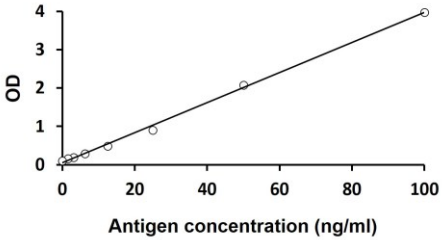
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples and zero controls into appropriate wells.
3. Add 100 µl of Assay buffer into all wells immediately. Mix thoroughly by gently shaking or tapping the plate. Cover the plate and incubate for 1 hour at 37°C.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of 1X HRP-antibody conjugate into each well. Mix thoroughly by gently shaking or tapping the plate. Cover wells and incubate for 1 hour at 37°C.
6. Aspirate each well and wash as step 4.
7. Add 100 µl of TMB substrate to each well. Incubate for 10 minutes at 37°C in dark. Substrate will change from colorless to different strengths of blue.
8. Add 50 µl of Stop solution to each well. The color of the solution should change from blue to yellow. Mix thoroughly by gently shaking the plate.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted or concentrated, the concentration read from the standard curve must be further converted by the appropriate dilution or concentration factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

Standard range: 1.56 – 100 ng/ml

Minimum Detectable Concentration: 0.5 ng/ml

Precision:

Intra-assay: 6 %

Inter-assay: 6 %

Recovery:

87-120.7 %

Specificity

The ELISA kit reacts to DENV type 1-4 NS1 protein and it does not cross-react to ZIKV NS1 protein.

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