TRUSTwell®

# Dengue Ag (NS1) ELISA Kit

**REF**

**AE0312**

**IVD**

- 96-well ELISA Test for the qualitative detection of dengue NS1 antigen (DEN1, 2, 3, 4) in human serum or plasma
- Store at 2- 8°C upon receipt

## INTENDED USE

The TRUSTwell Dengue Ag (NS1) ELISA Kit is a solid-phase enzyme-linked immunosorbent assay for the qualitative detection of dengue NS1 antigen (DEN1, 2, 3, 4) in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of an acute infection with dengue viruses.

## INTRODUCTION

Dengue virus is an enveloped, single-stranded, positive-sense RNA virus that comprises four related but distinct serotypes (DEN1, 2, 3, and 4). The virus is transmitted by mosquitoes of the daytime-biting Stegomyia family, principally Aedes aegypti and Aedes albopictus. Almost 4 billion people are at risk for dengue infection[1]. It has been estimated that 100-400 million cases of dengue infections can occur annually on a worldwide basis[2].

Dengue NS1 antigen is released into the blood during viral replication in an infected patient, and is detectable from the first day after the onset of fever up to Day 9[3,4]. NS1 antigen can be identified before the formation of antibodies, thus making it a beneficial biomarker for early detection of dengue infection, allowing for prompt management of dengue fever[5,6]. Immune responses to a dengue infection vary depending on the immune status of the patient. During a primary infection, IgM anti-dengue virus starts to appear approximately 4-6 days after the onset of fever, peaks after approximately two weeks, and remains in circulation for about 2-3 months4. IgG anti-dengue virus levels begin to increase slowly, peak around 14-21 days, and then decrease to low levels, persisting for the duration of life[6]. During secondary infection, NS1 antigen can be detected in patients for up to 9 days after the onset of illness. However it was reported that NS1 detection could be compromised by pre-existing anti-dengue IgG antibodies[7].

The TRUSTwell Dengue Ag (NS1) ELISA Kit utilizes pairs of specific polyclonal and monoclonal anti-dengue antibodies for the detection of all four serotypes of dengue NS1 antigen (DEN1, 2, 3, 4) in human serum or plasma.

## TEST PRINCIPLE

TRUSTwell Dengue Ag (NS1) ELISA Kit is a solid-phase enzyme-linked immunosorbent assay based on the principle of the antibody sandwich technique for the detection of dengue NS1 antigen in human serum or plasma.

The TRUSTwell Dengue Ag (NS1) ELISA Kit is composed of two key components:

1. Solid microwells pre-coated with rabbit anti-pan dengue NS1 antibody,
2. Liquid conjugates composed of monoclonal antibodies recognize NS1 antigen from DEN1, 2, 3 and 4 conjugated with horseradish peroxidase (HRP-anti-dengue NS1 Conjugates).

During the assay, the test specimen is first incubated in the coated microwell. The dengue NS1 antigen, if present in the specimen, binds to the antibody coated on the microwell surface, and any unbound specimen is then removed by a wash step. During a second incubation with the HRP-anti-dengue NS1 conjugates, the dengue NS1 antigen absorbed on the surface of microwell binds to antibody in the HRP conjugate, forming a conjugate complex. Unbound conjugates are then removed by washing. After addition of the TMB substrate, the presence of the conjugate complex is shown by development of a blue color resulting from a reaction between the enzyme and substrate. This reaction is then quenched by addition of the Stop Solution, and the absorbance value for each microwell is determined using a spectrophotometer at 450/620-690 nm.

## MATERIALS AND REAGENTS

**Materials and reagents provided with the kit:**

| Item | Description | Quantity | Catalog |
|---|---|---|---|
| 1 | Anti-dengue NS1 Ab Coated Microwells | 8 wells x 12 strips | AE0312W |
| 2 | HRP-anti-dengue NS1 Conjugate | 12 mL | AE0312H |
| 3 | Dengue (NS1) Ag Positive Control | 0.75 mL | AE0312P |
| 4 | Dengue (NS1) Ag Negative Control | 0.75 mL | AE0312N |
| 5 | Wash Buffer (30X Concentrate) | 20 mL | AWE3000 |
| 6 | Sample Diluent | 12 mL | AE0312SD |
| 7 | TMB Substrate A | 6 mL | ATME2000A |
| 8 | TMB Substrate B | 6 mL | ATME2000B |
| 9 | Stop Solution | 12 mL | ASE1000 |
| 10 | ELISA working Sheet | 2 Nos | AE0001ES |
| 11 | Product Insert | 1 No | PI-AE0312 |
| 12 | Sealant | 3 Nos | N/A |
| 13 | Dessicant | 3 Nos | N/A |

**Materials and reagents required but not provided in the kit**

1. Pipette capable of delivering 50 µL and 100 µL volumes
2. Microplate reader with a bandwidth of 10 nm or less and an optical density
3. range of 0-3 OD or greater at 450 nm wavelength is acceptable
4. Absorbent paper for blotting the microwells
5. Timer
6. Distilled or de-ionized water

## STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Reseal the microwells after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Place unused wells in the resealable bag provided and return to 2-8°C. Once opened, the kit is stable for 8 weeks at 2-8°C, or until the labeled expiration date, whichever is earlier.

## WARNING AND PRECAUTIONS

***For in Vitro Diagnostic Use***

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
2. Do not use expired kits.
3. Bring all reagents to room temperature (20-25°C) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Do not use serum derived from hemolyzed blood specimens for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mouth. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as biohazardous waste.
10. At the beginning of each incubation, and after adding Stop Solution, gently rock the microwells to ensure thorough mixing. Avoid the formation of air bubbles which results in inaccurate absorbance values. Avoid splashing liquid while rocking or shaking the wells
11. Do not allow the microwells to dry between the end of the washing operation and the reagent distribution.
12. The enzyme-substrate reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or TMB Substrate.
13. The enzyme-substrate is temperature dependent. Ensure that the room temperature for TMB incubation falls between 20-25˚C.
14. The TMB Substrate must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate B must be stored in the dark.
15. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate.
16. The wash procedure is critical. Wells must be aspirated completely before adding the Wash buffer or liquid reagents. Automatic washers must be validated with the test kit prior to use. Insufficient washing will result in poor precision and falsely elevated absorbance values.
17. Microplate reader must be calibrated per manufacturer's instruction to ensure accurate determination of absorbance. Non-calibrated reader often leads to invalid test results.
18. Avoid exposure to strong light during color development.

## SPECIMEN COLLECTION AND PREPARATION

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, refrigerate at 2-8°C. If a storage period greater than three days is anticipated, the specimen 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.
4. Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assay.
5. Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

## PREPARATION OF THE REAGENTS

1. Bring all reagents, controls to room temperature (20-25°C).
2. **Preparation of working Wash Buffer**
   Warm up the concentrated Wash Buffer to 37°C to dissolve the precipitant if it appears. Dilute concentrated Wash Buffer 30 fold with water as follows:

| Plate | DI Water | 30x Wash Buffer | Final Volume |
|---|---|---|---|
| **1 strip** | 58 mL | 2.0 mL | 60 mL |
| **2 strip** | 116 mL | 4.0 mL | 120 mL |
| **3 strip** | 174 mL | 6.0 mL | 180 mL |
| **4 strip** | 232 mL | 8.0 mL | 240 mL |

   The diluted wash buffer can be stored at 2-8°C for up to 3 days.
3. Mix each reagent before adding to the test wells.
4. Determine the number of strips needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls should be run in duplicate to ensure accuracy.

## ASSAY PROCEDURE

1. Remove the desired number of strips and secure them in the microwell frame. Place unused strips into the resealable bag and seal for later use.
2. Add specimens to the plate according to the designation on the ELISA Working Sheet:
   - 2.1 Blank Wells: Do not add any reagents.
   - 2.2 Control wells: Add 100 µL of Positive, Negative Control into the designated control wells, respectively
   - 2.3 Test wells: Add 50 µL of sample diluent and 50 µL of test specimens to each test well, respectively.
   - 2.4 Gently shake the wells for 20 seconds, and then cover the plate with a sealer.
3. Incubate the wells at 37°C for 60 minutes.
4. Wash Step (Can be performed manually or with automated washing):
   **Manual washing:** Carefully remove the incubation mixture by disposing the solution into a waste container. Fill each well with 350 µL diluted wash buffer and shake gently for 20-30 seconds. Discard the wash solution completely. Repeat 4 more times. After completing the last wash step, tap the plate on absorbent paper to remove residual liquid.
   **Automatic washing:** Automatic plate washer must be calibrated to ensure efficient washing. Aspirate incubation mixture from all wells completely. Fill each well with 350 µL diluted wash buffer and soak for 20-30 seconds. Aspirate all wells completely. Repeat 4 more times.
5. Pipette 100 µL of HRP-anti-dengue NS1 Conjugate into each well, except the Blank Well. Gently shake the microwells to ensure thorough mixing and then cover the plate with a sealer.
6. Incubate at 37°C for 60 minutes.
7. Wash the plate as described in step 4.
8. Add 50 µL of TMB Substrate A and 50 µL of TMB Substrate B into each well including the Blank Well. Gently shake the microwells for 20 seconds to ensure thorough mixing and then cover the plate with a sealer.

9. Incubate at room temperature (20-25°C) in dark for 15 minutes.
10. Stop the reaction by adding 100 µL Stop Solution into each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color completely changes to a color yellow.**
11. Set the microplate reader wavelength at 450 nm. Measure the absorbance (OD) of each well against the Blank Well within 30 minutes after adding Stop Solution. A filter of 620-690 nm can be used as a reference wavelength to optimize the assay result.

| Flow chart of assay procedure | | |
|---|---|---|
| 1. | Secure strips in microwell frame | Number of strips |
| 2. | Add NC, PC<br>Gently shake | 100 µL |
| 3. | Add sample diluent and specimens.<br>Gently shake | 50 µL + 50 µL<br>20 seconds |
| 4. | Incubate | 37°C,<br>60 minutes |
| 5. | Wash: manual or automatic | 5 times |
| 6. | Add HRP-anti-dengue NS1 Conjugate,<br>except Blank Wells | 100 µL |
| 7. | Incubate | 37°C,<br>60 minutes |
| 8. | Wash: manual or automatic | 5 times |
| 9. | Add TMB Substrate A and B<br>Gently shake | 50 µL + 50 µL<br>20 seconds |
| 10. | Incubate in dark | RT (20-25°C)<br>15 minutes |
| 11. | Add Stop Solution.<br>Gently shake | 100 µL,<br>30 seconds |
| 12. | Read result | 450/620-690 nm<br>within 30 minutes |

## INTERPRETATION OF RESULTS

**A.Set up the cut-off value**

The cut-off value = 0.20 + N

N: Mean OD of the negative control. Use N=0.10 for calculation of the cut-off value if the mean OD is less than 0.10.

**B. Calculation of specimen OD ratio**

Calculate an OD ratio for each specimen by dividing its OD value by the cut-off value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut-off value}}$$

**C. Assay Validation**

The mean OD value of the positive controls should be ≥ 0.8

The mean OD value of the negative controls should be ≤ 0.2

Check the assay procedure including incubation time and temperature and repeat assay if above criteria is not met.

**D. Interpretation of the results**

**Specimen OD ratio:** *Negative < 1.0; Positive ≥ 1.0*

If above specification are not met, the assay is Invalid. Check the assay procedure including incubation time and temperature and repeat assay.

1. A negative result indicates that there is no detectable dengue NS1 in the specimen.
2. Specimens with OD ratio > 1.0 are initially considered to be positive by the TRUSTwell Dengue Ag (NS1) ELISA Kit. Results should be used in conjunction with clinical findings to diagnose dengue infection.
3 Results just below the cut-off value (lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to re-test in duplicate the corresponding specimens when it is applicable).
3. If after retesting the absorbance of one of the duplicates is equal to or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the TRUSTwell Dengue Ag (NS1) ELISA Kit, subject to the limitations of the procedure, described below.

   If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non-repeatable and the specimen is considered to be negative with the TRUSTwell Dengue Ag (NS1) ELISA Kit.

   Non-repeatable reactions are often caused by:

- Inadequate microwell washing
- Contamination of negative specimens by serum or plasma with a high concentration of NS1 antigen
- Contamination of the TMB Substrate by oxidizing agents (bleach, metal ions, etc.)
- Contamination of the Stop Solution

## PERFORMANCE CHARACTERISTICS

**1) Analytical Sensitivity**

The TRUSTwell Dengue Ag (NS1) ELISA Kit can detect recombinant dengue type 2 NS1 antigen at a level as low as 0.3 ng/mL, when spiked into negative specimens.

**2) Clinical Performance**

A total of 527 specimens were collected from susceptible subjects and tested by TRUSTwell Dengue Ag (NS1) ELISA Kit and by a commercial leading brand EIA. Discordant results were confirmed by PCR. The clinical performance of the TRUSTwell Dengue Ag (NS1) ELISA Kit for all subjects is shown in the following table:

| | TRUSTwell Dengue Ag (NS1) ELISA Kit Kit | | |
|---|---|---|---|
| **Reference ELISA** | **Positive** | **Negative** | **CV** |
| **Positive** | 123 | 0 | 123 |
| **Negative** | 3 | 401 | 404 |
| **Total** | 126 | 401 | 527 |

Relative Sensitivity: 100% (95% CI: 96.97%-100%);Specificity: 99.26% (95% CI: 97.84%-99.75%) Overall agreement: 99.43% (95% CI: 98.34-99.81%)

**3) Precision**

a. Intra-Assay Precision was determined by assaying 15 replicates of three specimens (negative, low positive, and high positive). The mean, standard deviation (SD) and coefficient of variation (CV) were calculated and the results are listed in the following table:

| Panel | N | OD | SD | CV |
|---|---|---|---|---|
| Negative | 15 | 0.193 | 0.017 | 8.82% |
| Low positive | 15 | 0.803 | 0.063 | 7.81% |
| High positive | 15 | 1.538 | 0.109 | 7.10% |

b. Inter-Assay Precision was determined by assaying three specimens (negative, low positive, high positive) in 10 separate runs. The mean, SD, and CV were calculated and the results are listed in the following table:

| Panel | Runs | OD | SD | CV |
|---|---|---|---|---|
| **Negative** | 10 | 0.159 | 0.011 | 6.81% |
| **Low positive** | 10 | 0.846 | 0.040 | 4.72% |
| **High positive** | 10 | 1.41 | 0.058 | 4.12% |

**3) Cross-reactivity**

No false positive Dengue Ag (NS1) ELISA results were observed on 3-10 positive specimens from each of the following disease states or special conditions, respectively:

| | | | | |
|---|---|---|---|---|
| HCV | HBsAg | HIV | H. pylori | Malaria |
| Syphilis | ANA | HAMA | RF (up to 8,400 IU/mL) | |

**4) Hook effect**

No hook effect was observed at the concentration of the Dengue NS1 Ag up to 0.1 mg/mL.

**5) Interference**

Common substances (such as pain and fever medication and blood components) may affect the performance of the TRUSTwell Dengue Ag (NS1) ELISA Kit. Interference was studied by spiking these substances into 3 dengue antigen clinical specimens: negative, low positive and high positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the TRUSTwell Dengue Ag (NS1) ELISA Kit.

List of potentially interfering substances and concentrations tested:

1. Salicylic acid 4.34 mmol/L
2. Sodium citrate 1.3 %
3. Creatinine 0.5 mmol/L
4. EDTA 3.4 µmol/L
5. Glucose 55 mmol/L
6. Heparin 3,000 U/L
7. Bilirubin 10 mg/dL

## LIMITATIONS OF THE TEST

1. The Assay Procedure and the Interpretation of Results must be followed closely when testing for the presence of dengue NS1 antigen in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTwell Dengue Ag (NS1) ELISA Kit is limited to the qualitative detection of dengue virus NS1 antigen in human serum or plasma. The intensity of the color does not have linear correlation with the dengue NS1 antigen concentration in the specimen.
3. The TRUSTwell Dengue Ag (NS1) ELISA Kit cannot be used to differentiate between primary or secondary dengue infections. No information on the dengue serotype(s) present in a specimen can be provided with this test.
4. A negative result for an individual subject indicates absence of detectable dengue NS1 antigen. However, a negative test result does not preclude the possibility of exposure to or infection with dengue virus.
5. A negative result can occur if the quantity of the dengue NS1 antigen present in the specimen is below the detection limits of the assay, or the antigen detected is not present during the stage of disease in which a specimen is collected.
6. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect results.
7. Infection may progress rapidly. If the symptoms persist, while the result from the TRUSTwell Dengue Ag (NS1) ELISA Kit is negative, it is recommended to test with an alternative test method.
8. Any use or interpretation of this test's results must also rely on other clinical findings and the professional judgment of health care providers.

## REFERENCES


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7. Dussart P. et al. Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. Clin. Vaccine Immunol. 13, 1185–1189 (2006).
8. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements


**Index of Symbols**

| | | |
|---|---|---|
| Consult instructions for use | REF Catalogue number | Use-by date |
| IVD For *in vitro* diagnostic use only | LOT Batch code | Tests per kit |
| 2°C 8°C Temperature limit 2-8°C | Do not re-use | Keep dry |
| Manufacturer | Date of manufacture | If device is non-sterile |
| Do not use if package is damaged | Keep away from sunlight | Warnings / Precautions |


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