



INTENDED USE

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

INTRODUCTION

Dengue virus is an enveloped, single-stranded, positive-sense RNA virus that belongs to the *Flaviviridae* family and can be classified into four distinct serotypes (DEN1, 2, 3, 4). The virus is transmitted by daytime-biting mosquitoes, principally *Aedes aegypti* and *Aedes albopictus*. Currently, more than 2.5 billion people living in tropical and subtropical areas of Asia, Africa, Australia, and the Americas are at risk for dengue infection. An estimated 67-136 million cases of dengue fever and 20,000 deaths occur annually on a worldwide basis¹⁻³.

Serological detection is a common method for the diagnosis of infection with dengue virus. 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 months^{4,5}. IgG anti-dengue virus levels increase slowly, peak around 14-21 days, and then decrease to low levels, persisting for life⁶.

During secondary infection, IgM antibodies increase simultaneously with, or following the IgG secondary antibodies, which is a strong and rapid response, and can be detected as early as three days after the onset of symptoms. IgM antibody levels occur, in general, at a lower titer than that of IgG^{5,6}. Importantly, during secondary infection, IgM antibody levels are significantly lower in comparison with primary infection⁶.

The Dengue IgM ELISA Kit uses recombinant dengue virus antigens to specifically detect IgM antibodies for all four dengue virus serotypes (DEN1, 2, 3 and 4).

TEST PRINCIPLE

The Dengue IgM ELISA Kit is a solid-phase enzyme-linked immunosorbent assay based on the principle of the capture immunoassay methodology for the detection of IgM anti-dengue virus in human serum or plasma.

The Dengue IgM ELISA Kit is composed of two key components:

- 1) Solid microwells pre-coated with mouse monoclonal anti-human IgM antibody.
- 2) Conjugate composed of biotinylated dengue antigens and avidin HRP-conjugates.

During the assay, the test specimen is first incubated in the coated microwell. IgM anti-dengue, if present in the specimen, binds to the anti-human IgM antibodies coated on the microwell surface, and any unbound specimen is then removed by a wash step. During a second incubation with Biotinylated dengue antigens, the IgM anti-dengue absorbed on the surface of microwell binds with dengue antigen, forming a complex. Unbound antigens are then removed by washing. After addition of Streptavidin HRP conjugate, it binds with biotinylated antigen and forming complex with HRP conjugate. 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-human IgM Coated Microwells	8 wells x 12 strips	AE0310W
2	Biotinylated Ag mix(251X concentrate)	100 µL	AE0310Ag
3	HRP conjugates	12 mL	AE0310H
4	Dengue IgM Positive Control	0.75 mL	AE0310P
5	Dengue IgM Negative Control	0.75 mL	AE0310N

6	Sample Diluent	90 mL	AE0310SD
7	Wash Buffer (30X Concentrate)	30 mL	AWE3000
8	TMB Substrate A	6 mL	ATME2000A
9	TMB Substrate B	6 mL	ATME2000B
10	Stop Solution	12 mL	ASE1000
11	ELISA Working Sheet	2	AE0001ES
12	Product Insert	1	PI-AE0310
13	Microplate Sealers	3	N/A

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 5 µL, 50 µL and 100 µL volumes with a precision better than 98.5%
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450 nm wavelength is acceptable
3. Absorbent paper for blotting the microwells
4. Timer
5. Distilled or de-ionized water

STORAGE AND STABILITY

All reagents except Dengue Antigen Mix and concentrated Wash Buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Ensure that the reagents are brought to room temperature before opening. Return all reagents requiring storage at 2-8°C to refrigeration immediately after use. Re-seal the unused microwells in provided Ziploc bag with desiccant. All reagents are stable through the expiration date printed on the label if not opened. Once opened, the kit is stable for 8 weeks at 2-8°C, or until the labeled expiration date, whichever is earlier.

SPECIMEN COLLECTION AND PREPARATION

1. Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
2. This Test is designed for use with serum or plasma specimens without additives only.
3. If a specimen is not tested immediately, refrigerate at 2-8°C for up to 3 days. For storage longer than 3 days, the specimen should be frozen at -20°C. Avoid multiple freeze-thaw cycles. If a specimen is to be shipped, pack in compliance with federal regulations covering the transportation of etiologic agents.
4. Specimens containing precipitates may give inconsistent test results. Clarify such specimens by centrifugation prior to performing the assay.
5. Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS PRIOR TO ASSAYING

1. Bring all reagents and controls to room temperature (20-25°C).
2. Determine the volumes of antigen working solution and wash buffer required for the assay. Each strip of microwells requires 1000 µL of antigen working solution and 60mL of working wash buffer.
3. **Preparation of antigen working solution (1X):**

Plate	Sample Diluent	Biotinylated Ag mix (251X concentrate)	Final volume
1 strip	1000 µL	4 µL	1004 µL
2 strips	2000 µL	8 µL	2008 µL
3 strips	3000 µL	12 µL	3012 µL
6 strips	6000 µL	24 µL	6024 µL

4. **Preparation of specimens:**
Dilute the patient specimens 100 fold with Sample Diluent: e.g. to 5 µL of specimen, add 495 µL Sample Diluent.
5. **Preparation of working Wash Buffer:**
If precipitants are visible, warm the Wash Buffer to 37°C. Dilute concentrated Wash Buffer 30 fold with distilled or de-ionized water as follows:

Plate	DI water	Wash buffer (30X)	Final volume
1 strip	58 mL	2 mL	60 mL
2 strips	116 mL	4 mL	120 mL
3 strips	174 mL	6 mL	180 mL
6 strips	348 mL	12 mL	360 mL

6. Mix each reagent before adding it to the test wells.
7. Determine the number of microwells needed and mark the ELISA working sheet with the appropriate information. Run positive and negative controls in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Remove the desired number of strips and secure them in the microwell frame. Reseal un-used strips in Ziploc bag.
2. Add specimens according to the designation on the ELISA Working Sheet:
2.1: **Blank well:** Do not add specimens or control.

2.2: Control wells: Add 100 µL of Positive, Negative Control into the designated control wells, respectively.

2.3 Test wells: Add 100 µL diluted patient specimen into each designated test well.

2.4 Gently rock the plate for 20 seconds, and then cover the plate with the sealer.

3. Incubate the plate at 37°C for 30 minutes.

4. Wash to remove unbound materials:

Manual washing: Carefully remove the incubation mixture by disposing of solution into a waste container. Fill each well with 350 µL diluted wash buffer and rock 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.

Automated washing: Automatic plate washer must be calibrated to ensure efficient washing. Fill each well with 350 µL diluted wash buffer and soak for 20-30 seconds. Aspirate all wells completely. Repeat 4 more times.

5. Add 100 µL prepared antigen working solution(1X) into each well except the blank well. Gently rock the microwells for 20 seconds to ensure thorough mixing.

6. Cover the wells and incubate at 37 °C for 45 minutes.

7. Wash the plate 5 times as described in step 4.

8. Add 100 µL of HRP conjugate into each well except the blank well.

9. Cover the wells and incubate at 37°C for 20 minutes

10. Wash the plate 5 times as described in step 4.

11. Add 50 µL TMB Substrate A and 50 µL TMB Substrate B into each well, including the Blank Well. Gently rock the microwells for 20 seconds to ensure thorough mixing.

12. Incubate at room temperature (20-25°C) in the dark for 15 minutes.

13. Stop the reaction by adding 100 µL Stop Solution to each well, including the Blank Well. Gently rock for 20 seconds. Pipette the Stop Solution in the same sequence as substrate addition. **It is important to make sure that all the blue color completely changes to the color yellow.**

14. Set the microplate reader wavelength at 450 nm. Measure the absorbance (OD) of each well against the blank well within 30minutes 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 & diluted specimens. Gently rock		100 µL 20 seconds
3.	Incubate		37°C, 30 minutes
4.	Wash: manual or automatic		5 times
5.	Add antigen working solution. Gently rock		100 µL 20 seconds
6.	Incubate		37°C, 45 minutes
7.	Wash: manual or automatic		5 times
8.	Add HRP conjugate		100 µL
9.	Incubate		37°C, 20 minutes
10.	Wash: manual or automatic		5 times
11.	Add TMB Substrate A and TMB Substrate B		50 µL + 50 µL 20 seconds
12.	Incubate in dark		RT (20-25°C) 15 minutes
13.	Add Stop Solution.Gently rock		100 µL, 20 seconds
14.	Read result		450/620-690 nm within 30 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cut-off value = 0.25 + NC

NC: Mean OD of the Negative Control.

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 ≥ 0.50

The mean OD value of the negative controls should be ≤ 0.13

Check the assay procedure including incubation time and temperature and repeat assay if above conditions are not met.

D. Interpretation of the results

Specimen OD ratio

Negative	< 1.0
Positive	≥ 1.0

1. A negative result indicates that there is no detectable IgM anti-dengue antibody in the specimen.
2. 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. Specimens with OD ratio ≥ 1.0 are initially considered to be positive by the Dengue IgM ELISA Kit. They should be retested in duplicate before a final interpretation is made.

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 Dengue IgM ELISA Kit.

Non-repeatable reactions are often caused by:

- Inadequate microwell washing.
- Contamination of negative specimens by serum or plasma with a high antibody titer.
- Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.).
- Contamination of the Stop Solution.

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 Dengue IgM ELISA Kit, subject to the limitations of the procedure, described below.

PERFORMANCE CHARACTERISTICS

1. Accuracy of Detection

A total of 446 patient specimens were collected from susceptible subjects and tested by Dengue IgM ELISA Kit and by a commercial leading brand EIA. Comparison for all subjects is shown in the following table:

Reference EIA	Dengue IgM ELISA		
	Positive	Negative	Total
Positive	111	10	121
Negative	15	310	325
Total	126	320	446

Relative Sensitivity: 91.7% (95% Confidence Interval = 85.3 – 95.9%)

Relative Specificity: 95.4% (95% Confidence Interval = 92.5 – 97.4%)

Overall agreement: 94.4% (95% Confidence Interval = 91.8 – 96.3%)

2. Precision

- Intra-assay Precision was determined by assaying 20 replicates of three patient specimens.

Sample	N	OD	SD	CV
Negative	20	0.142	0.012	8.72%
High Positive	20	1.558	0.078	4.99%
Low Positive	20	0.444	0.025	5.63%

- Inter-assay Precision was determined by assaying three patient specimens in 10 separate runs. Data were analyzed by ANOVA (analysis of variance).

Specimen	Runs	OD	SD	CV
Negative	10	0.144	0.010	7.00%
High Positive	10	1.591	0.054	3.44%
Low Positive	10	0.452	0.017	3.90%

3. Cross-Reactivity

No false positive Dengue IgM ELISA test results were observed on 2-10 positive specimens from each of the following disease states or special conditions, respectively:

CHIK	HCV	HBsAg	HIV
Malaria	Syphilis	Typhoid	

4. Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the Dengue IgM ELISA Kit. Interference was studied by spiking these substances into 3 dengue IgM 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 Dengue IgM ELISA Kit.

List of potentially interfering substances and concentrations tested:

1. Salicylic acid	4.34 mmol/L	5. Glucose	55 mmol/L
2. Sodium citrate	1.3 %	6. Heparin	3,000 U/L
3. Creatinine	442 µmol/L	7. Bilirubin	10 mg/dL
4. EDTA	3.4 µmol/L		

WARNING AND PRECAUTIONS

For in Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert may lead to inaccurate test results.
2. Bring all reagents to room temperature (20-25°C) before use.
3. Do not use expired test kits.
4. Do not use the components from any other type of test kit as a substitute for the components in this kit.
5. Do not use 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. The human serum materials used for the preparation of controls have been tested and found non-reactive with antibodies to HIV-1 & 2, HCV, and HBsAg. However, 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 bio-hazardous waste.
10. At the beginning of each incubation, and after the addition of Stop Solution, gently rock the microwells for 20 seconds to ensure thorough mixing and even distribution. 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. Do not allow any metal element to come into contact with the conjugate or substrate solution.
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 and working step. Never use the specimen container to distribute dispense conjugate and TMB Substrate.
16. The wash procedure is critical. Wells must be aspirated completely before adding the wash buffer or liquid reagents. Automatic washer must be validated with Dengue IgM ELISA 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 readers often lead to invalid test results.
18. Avoid exposure of the wells to strong light during color development.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Results must be followed closely when testing for the presence of antibodies to dengue virus in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.

2. The Dengue IgM ELISA Kit is limited to the qualitative detection of IgM antibodies to dengue virus in human serum or plasma.
3. The Dengue IgM ELISA Kit cannot be used to differentiate if the infection is primary or secondary. However, it has been proposed that antibody ratios (IgG/IgM) can be used to distinguish between primary and secondary dengue infections⁵.
4. Serological cross reactivity with other flaviviruses is common (e.g., Japanese encephalitis, West Nile, yellow fever, etc.), therefore, it is possible that patients infected with these viruses may show some level of the reactivity with this test.
5. A negative result for an individual subject indicates absence of detectable dengue virus antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with dengue virus.
6. A negative result can occur if the titer of the dengue IgM antibodies present in the specimen is below the detection limit of the assay, or the IgM antibodies that are detected are not present during the stage of disease in which a specimen is collected.
7. Infection may progress rapidly. If the symptoms persist, while the result from the Dengue IgM ELISA Kit is negative, it is recommended to test with an alternative test method.
8. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect the expected results.
9. Any interpretation or use of this test result must also rely on other clinical findings as well as on the professional judgement of health care providers.

REFERENCES

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5. Halstead SB, Suaya JA, Shepard DS. The burden of dengue infection. Lancet, 2007; 369:1410-1411.
6. Peeling RW et al. Evaluation of diagnostic tests: dengue. Nature Reviews Microbiology 8, S30-S37. 2010.
7. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

Index of Symbols⁷

	Consult instructions for use		Batch code		Use-by date
	In vitro diagnostic medical device		Do not re-use		Keep dry
	Temperature limit 2-8 °C		Date of manufacture		Tests per kit
	Manufacturer		Caution		European Conformity
	Catalogue number		Non-sterile		Authorized Representative
	Do not use if package is damaged		Keep away from sunlight		