Human Dengue Virus IgM antibody ELISA Kit

Enzyme Immunoassay for the determination of Dengue Virus IgM in serum and plasma

Catalog number: ARG80539

Package: 96 wells

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INTRODUCTION

Dengue virus is a single-stranded RNA virus of about 50 nm in diameter belonging to the genus Flavivirus. Dengue and dengue hemorrhagic fever are caused by one of four closely related, but antigenically distinct, virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4). Infection with one of these serotypes does not provide cross protective immunity, so persons living in a dengue-endemic area can have four dengue infections during their lifetimes. The viruses are transmitted by Aedes aegypti, a domestic, day-biting mosquito that prefers to feed on humans. Infection with dengue viruses produces a spectrum of clinical illness ranging from a nonspecific viral syndrome to severe and fatal hemorrhagic disease. It is primarily a disease of the tropics; its global distribution is comparable to that of malaria, and an estimated 2.5 billion people live in areas at risk for epidemic transmission.- Globally, there are an estimated 50 to 100 million cases of dengue fever and several hundred thousand cases of dengue hemorrhagic fever.

- The case-fatality rate of DHF in most countries is about 5%; most fatal cases are among children and young adults.
- Important risk factors for DHF include the strain and serotype of the infecting virus, as well as the age, immune status, and
- Genetic predisposition of the patient.
- Risk groups: residents of or visitors to tropical urban areas.

Species	Disease	Symptoms	Mechanism of Infection
Dengue virus	Dengue Dengue hemorrhagic fever (DHF) or Breakbone fever	Sudden onset of fever, severe headache, myalgias and arthralgia leukopenia, thrombocytopenia and hemorrhagic manifestations	Transmission by mosquitos (Aedes aegypti)

The presence of virus resp. infection may be identified by

• Serology: Detection of antibodies by ELISA

Infection produces lifelong immunity, but the antigenically distinct serotypes do not provide cross-protective immunity, so a person can theoretically experience four dengue infections; a dengue vaccine is not available

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. Dengue Virus antigens has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Dengue virus antibody present is bound by the immobilized antigens. After washing away any unbound substances, an HRP-conjugated antibody specific for human IgM is added to each well and incubate. Following the washing of any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of antigen-antibody binding 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.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Calibrator A (Negative Control)	2ml	4°C
Calibrator B (Cut-off Standard)	3ml	4°C
Calibrator C (Weak Positive Control)	2ml	4°C
HRP-conjugated antibody	20ml (Ready-to-use)	4°C
Sample Diluent	100ml	4°C
20X Wash buffer	50ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (Optional: 620 nm as reference wavelength)
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- 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 2-8°C at all times. Avoid using reagents from different batches.
- If crystals are observed in the 20X Wash buffer, warm to 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 20-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- 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.

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 heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note:

1. If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
2. Heat inactivation of samples is not recommended.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute **20X** Wash buffer into **distilled water** to yield 1X Wash buffer. (e.g. 10 ml of 20X Wash buffer +190 ml of distilled water). Mix thoroughly by Use a magnetic stirrer. The diluted 1X wash buffer is stable for 5 days at room temperature (20-25 °C).
- **Patient sample**: Dilute patient sample **1:101** with **Sample diluent buffer** before assay, mix well. (e.g. 5 µl of serum + 500 µl of sample diluent buffer)

  Note: *The controls are ready-to-use and need not further dilution.*

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25 °C) 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 **controls, diluted samples (1:101)** into wells. Leave one well empty for the Substrate Blank. Cover the wells and incubate for **1h at 37°C**.
3. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes** (for automatic systems wash the wells for a total 5 washes). Wash by filling each well with **1× Wash Buffer (350 µl)** using a squirt bottle, manifold dispenser, or autowasher. Avoid overflows from the reaction wells. Keep the wash buffer in the wells for > 5 sec before removal. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µl** of **HRP-conjugated antibody** into each well (except for the Substrate Blank well). Cover wells and incubate for **30 minutes at RT**.
5. Aspirate each well and **wash as step 3**.
6. Add **100 µl** of **TMB Substrate Reagent** to each well (including Substrate Blank well). Incubate for **15 minutes** at **room temperature** in dark. A blue color occurs due to an enzymatic reaction.
7. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at **450 nm** (and reference filter **620 nm**) immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Adjust the ELISA Microtiterplate reader to zero using the Substrate Blank.
2. Calculate the average absorbance values for each set of controls and samples.
3. In order for an assay to be considered valid, the following criteria must be met:

   Substrate blank: Absorbance value **<0.1**

   Negative control: Absorbance value **<0.2 and <cut-off**

   Cut-off control: Absorbance value **0.15-1.3**

   Positive control: Absorbance value **>cut-off**

   If these criteria are not met, the test is not valid and must be repeated.
4. Calculation of Results

   The Cut-off is the mean absorbance value of the Cut-off Control determinations.

   Example: Absorbance value of Cut-off Control well 1 = 0.5

   absorbance value Cut-off control well 2 = 0.52

   Control mean absorbance = Cut-off = (0.5+0.52)/2 = 0.51

5. Results in Units [U]

   Units [U] = [Sample (mean) absorbance value x 10] / Cut-off

   Example: (1.2 x 10) / 0.51 = 23.5 U (Units)

   Note: Cut-off = (Cut-off X 10) / Cut-off = 10 U

6. Interpretation of results:

- The samples are considered positive if the absorbance value is higher than 10% over the cut-off.

- Samples with absorbance value of 10% above or below cut-off should be considered in the grey zone.

It is recommended to repeat test again 2-4 weeks later with fresh sample. If the results in the second test are again in the grey zone, the sample has to be considered negative.

- Samples are considered negative if the absorbance value is lower than 10% below the cut-off.

Summary:

	Unit	Note
Cut-off	10 U	-
Positive	> 11 U	Antibodies to Dengue Virus were detected.
Equivocal	9 – 11 U	Antibodies to Dengue Virus could not be detected clearly.
Negative	< 9 U	Antibodies to Dengue Virus could not be detected
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay precision is 2.96% and inter-assay precision is 10.9%.

Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 96.59% (95% confidence interval: 92.73%- 98.74%).

Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 91.84% (95% confidence interval: 80.4%- 97.73%).

Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.