Dengue Virus IgG ELISA Kit

rev 10/*18*

(Catalog # E4670-100, 96 assays; Store at 2-8^{0}C)

I. Introduction:

The mosquito-borne dengue viruses (serotype 1-4) cause dengue fever, a severe flu-like illness. The disease is prevalent in Third World tropical regions and spreading to sub-tropical developed countries - including the United States. WHO estimates that 50-80 million cases of dengue fever occur worldwide each year, including a potentially deadly form of the disease called dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Primary infection with dengue virus results in a self-limiting disease characterized by mild to high fever lasting 3 to 7 days, severe headache with pain behind the eyes, muscle and joint pain, rash and vomiting. Secondary infection is the more common form of the disease in many parts of Southeast Asia and South America. This form of the disease is more serious and can result in DHF and DSS. The major clinical symptoms can include high fever, hemorrhagic events, and circulatory failure, and the fatality rate can be as high as 40%. Early diagnosis of DSS is particularly important, as patients may die within 12 to 24 h if appropriate treatment is not administered. Primary dengue virus infection is characterized by elevations 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.

II. Application:

Detection of IgG antibody to Dengue Virus

III. Sample Type:

Human serum or plasma

IV. Kit Contents:

Components	E4670-100	Part No.
Microplate	12 strips x 8 wells	E4670-100-1
Sample Diluent	22 ml	E4670-100-2
Calibrator	1 ml	E4670-100-3
Positive Control	1 ml	E4670-100-4
Negative Control	1 ml	E4670-100-5
Enzyme conjugate	12 ml	E4670-100-6
TMB Substrate	12 ml	E4670-100-7
Stop Solution	12 ml	E4670-100-8
Wash Buffer (20X)	25 ml	E4670-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VI. Storage Conditions and Reagent Preparation:

Store kit at 2-8^{0}C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Buffer:** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

VII. Warning & Precautions:

- Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories."
- Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

VIII. Sample Preparation and Storage:

Collect blood specimens & separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 7 days. Store frozen at (-20°C) for up to six month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well.

IX. Assay Protocol:

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 50 µl of diluted sera, calibrator and controls into the appropriate wells.
4. Dispense 100 µl of Anti-human IgG Biotin Conjugate into all wells.
5. Carefully mix the wells contents, for 20 seconds using a plate shaker, and incubate for 60 minutes at room temperature.

6. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of Dengue enzyme conjugate into all wells.
8. Incubate for 60 minutes at room temperature.
9. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
10. Dispense 100 µl of TMB substrate and incubate for 15 minutes at room temperature.
11. Add 50 µl of stop solution.
12. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. of negative control should be less than 0.2, and less than the O.D. of calibrator.
2. The O.D. of positive control should be greater than the O.D. of calibrator.

INTERPRETATION

The following is intended as a guide to interpretation of Dengue virus IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Standard Units = (Mean absorbance value of sample / absorbance value of calibrator) * 10

STANDARD UNITS INTERPRETATION

< 0.9	No detectable antibody to IgG antibody by ELISA.
0.9 - 1.1	Borderline positive. Follow-up testing is recommend if clinically indicated.
> 1.1	Detectable antibody to IgG antibody by ELISA.

EXAMPLE OF TYPICAL RESULTS:

Calibrator mean OD = 0.34
Positive Control OD = 1.0
Negative Control OD = 0.05
Patient sample O.D. = 0.8
Standard Units: (0.8/ 0.34) *10 = 24

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

X. RELATED PRODUCTS:

- Dengue Virus IgM ELISA Kit (E4671)
- Recombinant Chikungunya Virus E1 (P1113)
- Recombinant Zika virus NS1 Protein (P1064)
- QuickDetect™ IgG (Human) ELISA Kit (E4475)
- Recombinant Zika Envelope Protein (P1063)

FOR RESEARCH USE ONLY! Not to be used on humans.