BOSTER

antibody and ELISA experts

Human Dengue Virus IgG ELISA Kit (Direct EIA)

Catalog number: EK7045

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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Human Dengue Virus IgG ELISA Kit (Direct EIA)

Catalog Number: EK7045

Size: 96 wells/kit

Sample Type: Serum and Plasma

Storage: Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

SUMMARY AND EXPLANATION

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.

PRINCIPLE OF THE TEST

Diluted patient serum and biotinylated anti-human IgG antibody reagent are added to the wells coated with Streptavidin. IgG antibodies in the patient serum binds to the x-h IgG antibody, and the complex is immobilized on the plate through streptavidin-biotin interactions. Unbound proteins are washed off through a washing step. Dengue antigen-HRP conjugate reagent is added into the wells. Dengue virus IgG specific antibody, if present, binds to the antigen. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

Kit Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2.Sample Diluent: 1 bottle (ready to use)	22 ml
3.Calibrator: Yellow Cap, 1 Vial (ready to use)	1ml
4.Positive Control: Red Cap, 1 vial (ready to use)	1ml
5.Negative Control: Blue Cap, 1 vial (ready to use)	1ml
6.Anti-human IgG Biotin Conjugate: 1 bottle (ready to use)	12ml
7.Dengue Enzyme conjugate: 1 bottle (ready to use)	12ml
8.TMB Substrate: 1 bottle (ready to use)	12ml
9. Stop Solution: 1 bottle (ready to use)	12ml
10.Wash concentrate 20X: 1 bottle	25ml

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Materials Required, but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

WARNINGS AND PRECAUTIONS

1. For research use only. Not for use in diagnostic procedures.
2. For laboratory use.
3. 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, 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" 1984
4. 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.
5. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
6. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
7. 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.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Typically, specimens may be refrigerated at 2-8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

REAGENT PREPARATION

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 (20-25°C).

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of specimens, by adding 10ul of the sample to 200ul of sample diluent. Mix well.
3. Dispense 50 ul of diluted sera, calibrator and controls into the appropriate wells.
4. Dispense 100 ul 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 ul of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 ul of Dengue enzyme conjugate into all wells
8. Incubate for 60 minutes at room temperature.

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9. Remove enzyme conjugate from all wells. Wash wells three times with 300 □l of 1X wash buffer. Blot on absorbance paper or paper towel.
10. Dispense 100 ul of TMB substrate and incubate for 15 minutes at room temperature.
11. Add 50 uL 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:
<9 No detectable antibody to Dengue IgG by ELISA
9-11 Borderline positive. Follow-up testing is recommended if clinically indicated
>11 Detectable antibody to Dengue IgG 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

LIMITATION 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.

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