Product Information

Dengue Virus IgM ELISA

Catalog Number SE120043
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

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 is 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 3–7 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 12–24 hours if appropriate treatment is not administered. Primary dengue virus infection is characterized by elevations in specific IgM antibody levels 3–5 days after the onset of symptoms; this generally persists for 30–60 days. IgG levels also become elevated after 10–14 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 1–2 days after the onset of symptoms.

The Dengue Virus IgM ELISA Kit is intended for the detection of IgM antibody to dengue virus in human serum or plasma.

The diluted serum (serum diluent contains sorbent to remove rheumatoid factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. 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 IgM specific antibody in the sample.

Components

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with Dengue antigen</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>Sample Diluent: 1 bottle (ready to use)</td>
<td>22 ml</td>
</tr>
<tr>
<td>Calibrator: yellow Cap. 1 Vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Positive Control: Red Cap. 1 vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Negative Control: Blue Cap. 1 vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Wash concentrate 20×: 1 bottle</td>
<td>25 ml</td>
</tr>
</tbody>
</table>

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper
Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Sample Preparation
1. Collect blood specimens and separate the serum.
2. 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.

20× Wash concentrate
Prepare 1× Wash Buffer by adding the contents of the bottle (25 ml, 20×) to 475 ml of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, controls, and serum samples be run in duplicate.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be >0.250.
2. The Ab index for Negative control should be <0.9.
3. The Ab Index for Positive control should be >1.2.

Bring all specimens and kit reagents to room temperature (18–26 °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 21-fold dilution of test samples, by adding 10 µl of the sample to 200 µl of Sample Diluent. Mix well.

Notes: To enhance sensitivity and specificity of this IgM test, the provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and has shown no interference with test results. It can be removed by centrifugation.

In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.

3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl of Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells 3 times with 300 µl of 1× wash buffer. Blot on absorbent paper or paper towel.
5. Dispense 100 µl of Enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove Enzyme conjugate from all wells. Wash wells 3 times with 300 µl of 1× wash buffer. Blot on absorbent paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µL of Stop Solution.
9. Read O.D. at 450 nm using ELISA reader within 15 minutes. A dual wavelength is recommended with reference filter of 600–650 nm.
Results
Calculations
1. Check Calibrator Factor (CF) value on the
calibrator bottle. This value might vary from lot to
lot. Make sure the value is checked on every kit.
2. Calculate the cut-off value: Calibrator OD \times
Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each
determination by dividing the O.D. value of each
sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 \times 0.5 = 0.400
Positive control O.D. = 1.2
Ab Index = 1.2/0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6/0.4 = 4.0

Notes: 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.

Lipemic or hemolyzed samples may cause erroneous
results.

Interpretation
The following is intended as a guide to interpretation of
Dengue virus IgM antibody index (Ab Index) test
results; each laboratory is encouraged to establish its
own criteria for test interpretation based on sample
populations encountered.

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

References
1. Pinheiro, F.P., and Corber, S.J., Global situation of
dengue and dengue haemorrhagic fever, and its
emergence in the Americas. World Health Stat Q,
2. Gubler, D.J., and Trent, D.W., Emergence of
epidemic dengue/dengue hemorrhagic fever as a
public health problem in the Americas. Infect.
3. Wu, S.J. et al., Evaluation of a dipstick enzyme-
linked immunosorbent assay for detection of
4. Lam, S.K., and Devine, P.L., Evaluation of capture
ELISA and rapid immunochromatographic test for
the determination of IgM and IgG antibodies
5. Rossi, C.A. et al., Laboratory diagnosis of acute
dengue fever during the United Nations Mission in

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