Dengue Virus IgM Capture DxSelect™ (OUS)

Product Code EL1500M
Rev. T

Indirect Enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of human IgM class antibodies to dengue virus

This package insert is for export only and not for distribution in the United States.

Outside of the United States:
For *in vitro* Diagnostic Use.

INTENDED USE

The Focus Diagnostics Dengue Virus IgM Capture DxSelect™ is a qualitative assay for the detection of human serum IgM antibodies to dengue virus (DV) infections to be used in support of the diagnosis of acute dengue virus infections in humans.

SUMMARY AND EXPLANATION OF TEST

Dengue fever (DF) is an acute, self limiting, viral disease that is characterized by fever, headache, body pains, rash, lymphadenopathy, and prostration. In its most severe form, dengue hemorrhagic fever (DHF), infected patients will experience severe fever and renal failure leading to the often fatal dengue shock syndrome (DSS).[1] It is estimated that approximately 2 billion people are at risk for DF world wide, and that over 1 million people per year are infected.[2] This, combined with the hundreds of thousands of cases of DSS, make dengue the most important arbovirus disease in the world.[2]

Dengue virus (DV) is a flavivirus and is closely related to the yellow fever virus, Japanese encephalitis virus and other group B Arboviruses. Members of this group possess single stranded RNA which is surrounded by an icosahedral nucleocapsid covered with a 10 nm deep lipid envelope.[3] There are 4 strains of dengue virus, each serologically distinct. Infection with 1 strain does not protect the host from infection by the others. In fact, one report suggests DHF and DSS occurs most commonly in individuals that have been infected previously by another strain.[4] The presence of circulating, non-neutralizing, cross-reactive DV antibody may act as an immune infection enhancement factor.[4] However, non-neutralizing, cross-reactive antibodies against other non-DV flaviviruses are not associated with immune infection enhancement.[4]

Dengue fever epidemics have been reported regularly throughout the world. The largest epidemics have occurred in the southern United States (1922 affecting over 1 million people), Australia (1925 and 1942), Greece (1927) and Japan (1942-1945).[2] Peruvian and CDC officials have reported a major DF outbreak that occurred between March to July 1990.[5] This epidemic centered around Iquitos, Peru, involving DV types 1 and 4, is the first laboratory confirmation of indigenous transmission of dengue in Peru.[5]

Dengue fever can be transmitted wherever the mosquito vectors, *Aedes aegypti* and *Aedes albopictus*, are found. *A. aegypti* is primarily localized to tropical and subtropical Americas and is indigenous to the southern part of the United States.[2] The primary vector for DF in Asia is *A. albopictus.* This mosquito has recently established itself in the United States as far north as central Illinois; however, DV transmission has not been associated with it to date.[2]

In most patients, suspected cases of DF are most rapidly diagnosed using serological methods. Traditionally, hemagglutination inhibition and plaque reduction neutralization have been used.[3] However, IgM Capture enzyme-linked immunosorbant assays (ELISA) have become the method of choice. In most individuals the IgM response to DF is strain specific and persists as long as 90 days.[6]

IgG antibody to dengue virus has been detected in patients as long as 60 years post infection.[7] Therefore, a single antibody determination should not be considered conclusive. However, the IgG ELISA is useful as an epidemiological tool when used to establish sero-prevalence. This information can also be valuable in studies designed to determine the role immune enhancement plays in DSS. As with most flaviviruses, IgG is highly cross-reactive among most members of the flavivirus group.

The Focus Diagnostics Dengue Virus IgM Capture DxSelect™ is intended for the detection of human serum IgM antibodies to DV types 1-4. This assay is specific for IgM and uses a flavivirus group monoclonal horseradish peroxidase conjugate with TMB as substrate. Each Capture Well is coated with antihuman IgM. The antigen solution contains equal proportions of inactivated DV types 1-4. The following virus strains are used: Type 1: TH-Sman; Type 2: TH-36, Type 3:H87; and Type 4: H241.

TEST PRINCIPLE

In the Focus Diagnostics Dengue Virus IgM Capture DxSelect™, the polystyrene microwells are coated with anti-human antibody specific for IgM (μ -chain). Diluted serum samples and controls are incubated in the wells, and IgM present in the sample binds to the anti-human antibody (IgM specific) in the wells. Nonspecific reactants are removed by washing. Dengue virus (DV) antigen is then added to the wells and incubated; and, if anti-DV IgM is present in the sample, the DV antigen binds to the anti-DV in the well. Unbound DV antigen is then removed by washing the well. Mouse anti-DV conjugated with horseradish peroxidase is then added to the wells and incubated; and, if DV antigen has been retained in the well by the anti-DV in the sample, the mouse anti DV: HRPO binds to the DV antigen in the wells. Excess conjugate is removed by washing. Enzyme substrate and chromogen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of optical density (OD) which is directly proportional to the amount of antigen-specific IgM present in the sample. Sample optical density readings are compared with reference cut-off OD readings to determine results.

Background Subtract Procedure

The Focus Diagnostics Dengue Virus IgM Capture DxSelect™ kit employs capture wells coated with anti-human IgM. Captured IgM with heterophilic antibody activity may directly bind the reporter reagent. One way to help mitigate these false reactions is through use of a background subtraction procedure. Published data have shown that low level reactive samples may benefit from implementation of background subtraction; however, each laboratory must define its own reflex testing algorithms for analytes measured by μ-capture ELISA.[11]

MATERIALS SUPPLIED

The Focus Diagnostics Dengue Virus IgM Capture DxSelect™ kit contains sufficient materials to perform 96 determinations. Allow the supplied reagents to warm to room temperature before use. All un-opened materials are stable at 2 to 8°C until the expiration date stated on the reagent label.

Antigen, Lyophilized | **REF** | **EL1522** | **Ag**

2 vials containing inactivated lyophilized dengue virus antigen (equal portions of DV types 1-4). Each 6 mL antigen vial will perform approximately 60 tests.

To reconstitute the antigen, add exactly 6 mL of the reconstitution solution provided. DO NOT USE DISTILLED WATER OR ANOTHER REAGENT OTHER THAN THE REAGENT PROVIDED FOR RECONSTITUTION. ASSAY RESULTS ARE INVALID IF ANY OTHER MATERIAL IS USED FOR RECONSTITUTION. Allow the antigen to rehydrate at room temperature for 1 hour prior to use: the antigen must be completely dissolved before use. Store the remaining antigen at 2 to 8°C for up to 30 days following reconstitution. If the remaining antigen will not be used within 30 days, aliquot and freeze at –70°C or colder. Thaw only once.

Antigen Reconstitution Solution, 13 mL | **REF** | **EL1523** | **SOLV** | **Ag**

1 vial containing cell culture water, surfactant and 0.1% sodium azide.

IgM Capture Wells, 96 wells | **REF** | **EL1521** | **Ab** | **IgM**

12 Eight-well polystyrene break-apart microwell strips on a frame. Each well is coated with anti-human IgM. Each strip may be broken down into individual wells for cost effective use. To avoid condensation, allow the antigen strips to warm to room temperature before opening the sealed packets.

IgM Conjugate, 16 mL | **REF** | **EL1502** | **CONJ** | **IgM**

1 vial of affinity-purified and peroxidase-conjugated mouse anti-flavivirus. Contains protein, buffer, and preservatives.

IgM Detectable Control, 0.30 mL | **REF** | **EL1515** | **CONTROL** | **>**

1 vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).

Non-Detectable Control, 0.30 mL | **REF** | **EL1512** | **CONTROL** | **<**

1 vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).

IgM Cut-Off Calibrator, 0.30 mL | **REF** | **EL1503** | **CONTROL** | **CAL**

1 vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).

Sample Diluent, 100 mL | **REF** | **EL1608** | **DIL** | **SPE**

1 vial of protein, surfactant, and preservatives in PBS.

10X Wash Buffer, 100 mL | **REF** | **EL0405** | **BUF** | **WASH**

1 vial of surfactant in PBS with preservatives. Prepare a 1X wash buffer solution before use.

To prepare a 1X wash buffer solution, mix 100 mL 10X Wash Buffer with 900 mL distilled (or deionized) water and rinse out any crystals. Use only the highest grade purified water for reconstitution of the wash buffer. It has been observed that some sources of deionized water contain materials which can interfere in the assay. Swirl until well mixed and all crystals are dissolved.

Substrate Reagent, 16 mL | **REF** | **EL0009** | **SUBS** | **TMB**

1 vial of tetramethylbenzidine (TMB) and horseradish peroxide in buffer. A dark blue color indicates contamination with peroxidase; and, if this occurs, use a fresh bottle. Ready to use.

Stop Reagent, 16 mL | **REF** | **EL0105** | **SOLN** | **STOP**

1 vial 1 M sulfuric acid.

Sealing Tape

3 sheets of sealing tape.

MATERIALS REQUIRED, BUT NOT SUPPLIED

1. Distilled water
2. 250 or 500 mL wash bottle
3. 1 L graduated cylinder
4. Test tubes for serum dilutions
5. 10 µL pipettors with disposable tips
6. 100 µL pipettors with disposable tips (100 µL 8- or 12-channel pipettor recommended for runs over 48 wells)
7. 1 mL pipet or dispenser
8. 5 mL pipet
9. Timer
10. Paper towels or absorbent paper
11. Sink
12. Vortex mixer or equivalent
13. ELISA plate spectrophotometer, wavelength = 450 nm

SHELF LIFE AND HANDLING

1. Kits and kit reagents are stable through the end of the month indicated by the label expiration date when stored at 2 to 8°C.
2. Do not use test kit or reagents beyond their expiration dates.
3. Do not expose reagents to strong light during storage or incubation.
4. Allow reagents to warm to room temperature before use.

WARNINGS AND PRECAUTIONS

1. This package insert is for export only and not for distribution in the United States. Outside of the United States this kit is for *in vitro* diagnostic use.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of with proper biohazard precautions. [9,10]

3. The lyophilized antigen contains 4 strains of inactivated DV; however, the reagent should be considered potentially infectious and handled accordingly.
4. The stop reagent contains sulfuric acid. Do not allow to contact skin or eyes. If exposed, flush with copious amounts of water.
5. Sodium azide at a concentration of 0.1% has been added to some reagents as an antibacterial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, controls should be discarded into sewerage only if diluted and flushed with large volumes of water. Use copper-free and lead-free drain systems where possible. Occasionally decontaminate the drains with 10% sodium hydroxide (CAUTION: caustic), allow to stand for 10 minutes, then flush with large volumes of water.
6. Do not substitute or mix reagents from different kit lots or from other manufacturers.
7. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
8. Cross-contamination of patient specimens can cause erroneous results. Add patient specimens and handle IgM Capture Well strips carefully to avoid mixing of sera from adjoining wells. Avoid contamination of the substrate reagent with traces of the enzyme conjugate.
9. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
10. Perform the assay at room temperature (approximate range 20 to 25°C).
11. Use proper pipetting techniques, maintaining the pipetting pattern throughout the procedure to ensure optimal and reproducible values.

SPECIMEN COLLECTION AND PREPARATION

Serum is the preferred specimen source. No attempt has been made to assess the assay's compatibility with other specimens. Hyperlipemic, heat-inactivated, hemolyzed, or contaminated sera may cause erroneous results; therefore, their use should be avoided.

Specimen Collection and Handling

Collect blood samples aseptically using approved venipuncture techniques by qualified personnel. Allow blood samples to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage at 2 to 8°C. If testing is to be delayed longer than 5 days, the sample should be frozen at –20°C or colder. Thaw and mix samples well prior to use.

Specimen, Controls and Calibrator Preparation

Dilute each specimen, control and calibrator 1:101 as follows: label tubes and dispense 1 mL of **Sample Diluent** into each labeled tube. Add **10 µL of specimen, control or calibrator** to each appropriate tube containing the 1mL Sample Diluent and mix well by vortex mixing.

TEST PROCEDURE

The Focus Diagnostics Dengue Virus IgM Capture DxSelect™ may be used in either of 2 ways. The classical CDC protocol uses an overnight capture antigen incubation step; or, as an alternative, the capture antigen incubation step can be shortened to 1 hour at room temperature.

1. Allow all reagents to warm to room temperature before use. Remove the IgM Capture Well packet from cold storage. To avoid condensation, allow micro-well strips to reach room temperature before opening the foil packet. If less than a full plate is to be used, return unused strips to the foil packet with desiccant and reseal completely. Store unused IgM Capture Wells at 2 to 8°C. (Note: At the end of the assay, retain the frame for use with the remaining strips.)
2. Prepare Antigen Solution (make sure reagent has reached room temperature). If the kit is being used for the first time, reconstitute sufficient antigen (see MATERIALS SUPPLIED, above).
3. Fill wells with 1X Wash Buffer solution (see MATERIALS SUPPLIED, above) and allow to soak for 5 minutes. Decant (or aspirate) the antigen wells and tap vigorously to remove Wash Buffer. Blot the emptied Capture Wells face down on clean paper towels or absorbent paper to remove residual Wash Buffer.
4. Dispense 100 µL of the Sample Diluent into the "blank" wells and 100 µL of each diluted specimen, control or calibrator (see Specimen, Controls, and Calibrator Preparation, above) into the appropriate wells. (Note: For runs with more than 48 wells it is recommended that 250 µL of each diluted sample first be added to a blank microtiter plate in the location corresponding to that in the ELISA wells. The samples can then be efficiently transferred into the Antigen Wells with a 100 µL 8- or 12-channel pipettor.)
5. Cover plates with sealing tape, and incubate for 60 ± 1 minute at room temperature (20 to 25°C).
6. Remove sealing tape, and empty the contents of the wells into a sink or a discard basin.
7. Fill each well with a gentle stream of 1X Wash Buffer solution then empty contents into a sink or a discard container.
8. Repeat wash (step 7) an additional 2 times, allow the last wash to soak for 5 minutes before decanting or aspirating.
9. Tap the Capture Wells vigorously to remove 1X Wash Buffer. Blot the emptied Capture Wells face down on clean paper towels or absorbent paper to remove residual 1X Wash Buffer.
10. Add 100 µL of the prepared (see step 2, above) Antigen Solution to all wells, using a 100 µL 8- or 12-channel pipettor.
11. Cover plates with sealing tape and incubate for 1 hour at room temperature (20 to 25°C).
12. Repeat wash steps 6 through 9.
13. Add 100 µL of IgM Conjugate to all wells, using a 100 µL 8- or 12-channel pipettor.
14. Cover plates with sealing tape, and incubate for 30 ± 1 minute at room temperature (20 to 25°C).
15. Repeat wash steps 6 through 9.
16. Add 100 µL of Substrate Reagent to all wells, using a 100 µL 8- or 12-channel pipettor. Begin incubation timing with the addition of Substrate Reagent to the first well. (Note: Never pour the substrate reagent into the same trough as was used for the conjugate.)
17. Incubate for 10 ± 1 minutes at room temperature (20 to 25°C).
18. Stop the reaction by adding 100 µL of Stop Reagent to all wells using a 100 µL 8- or 12-channel pipettor. Add the Stop Reagent in the same sequence and at the same pace as the Substrate was added. In antibody-positive wells, color should change from blue to yellow.
19. Gently blot the outside bottom of wells with a paper towel to remove droplets that may interfere with reading by the spectrophotometer. Do not rub with the paper towel as it may scratch the optical surface of the well. (Note: Large bubbles on the surface of the liquid may affect the OD readings.)
20. Measure the absorbance of each well within 1 hour of stopping the assay. Set the microwell spectrophotometer at a wavelength of 450 nm. Zero the instrument on the blank wells.

IgM Procedure (condensed version)

1. Dilute samples
   Serum samples and Controls: 1:101 in Sample Diluent. (e.g., 10 µL + 1000 µL)
2. Soak Wells for **5 minutes** with **1X Wash**, decant.
3. **100 µL** of **sample** and incubate for **60 minutes**, decant.
   Optional Background Subtract: 100 µL of diluted sample is added to each of two wells. One well adds DV antigen in Step 5, and the other well adds Sample Diluent in Step 5. **Important note:** Background subtract is one way to check for heterophile antibodies present in positive samples. Therefore, background subtract should not be performed unless the patient sample was initially positive.
4. Wash 3 times.
5. **100 µL** of **Antigen** and incubate for **60 minutes**, decant.
   Optional Background Subtract: Add 100 µL of Antigen to the "Ag" well, and add 100 µL Sample Diluent to the "SD" well, incubate for 60 minutes.
6. Wash 3 times.
7. **100 µL** of **Conjugate** and incubate for **30 minutes**, decant.
8. Wash 3 times.
9. **100 µL** of **Substrate Reagent** and incubate for **10 minutes**.
10. **100 µL** of **Stop Reagent**, read at λ = 450 nm.

Please see the **PROCEDURE** section for important details.

QUALITY CONTROL

Each plate run (or strips or wells from a single plate) must include the Cut-off Calibrator and 2 controls. If multiple plates are run, include the Cut-off Calibrator and 2 controls on each plate. It is recommended that until the user becomes familiar with the kit performance, all specimens, controls and the Cut-off Calibrator should be run in duplicate with the Cut-off Calibrator run twice for a total of 4 wells. If single wells are used, the Cut-off Calibrator should be run in triplicate. Include a minimum of 1 blank well (containing sample diluent only) for instrument calibration purposes.

The Cut-off Calibrator has been formulated to give the optimum differentiation between negative and positive sera. After subtracting out the blank wells, the target OD of the Cut-off Calibrator is approximately 0.250 OD units. Due to the varying laboratory conditions, the absorbance values may differ significantly. A range of 0.100 OD units to 0.500 OD units is typical. All replicate Cut-off Calibrator OD should be within 0.100 absorbance units from the mean. Kit performance and result integrity are a function of the index values for the controls, not the absolute OD of the Cut-off Calibrator alone.

Report results as index values relative to the Cut-off Calibrator. To calculate index values, divide specimen optical density (OD) values (corrected for blank readings) by the mean of the corrected Cut-off Calibrator absorbance values.

1. The Detectable Control index value should be between 1.5 and 3.5.
2. The Non-Detectable Control index value should be less than 0.8.

If the Calibrator or controls are not within these parameters, patient test results should be considered invalid and the assay repeated.

Calculation for the Background Subtract Results

Step 1. Calculate the **Index for the Controls** by dividing the Control OD by the Cut-off OD.

Index for the Controls (Cut-off and Control Index) = "Ag" OD/CO OD

Step 2. Calculate the **Index for Patient Samples** by first calculating the Net Patient OD (by subtracting the "SD" OD from the "Ag" OD), and then dividing the Net Patient OD by the Cut-off OD.

Net Patient OD = "Ag" OD - "SD" OD

Index for Patient Samples = Net Patient OD/CO OD

Step 3. Interpret using the ranges in the Interpretation section (e.g., negative < 1.00, and positive > 1.00).

Example Calculation for Optional Background Subtraction Results

ID	OD*		Step 1	Step 2		Step 3
	Ag OD	SD OD	Divide Control OD by Cut-off OD **Ag OD /CO OD = Index for Controls**	Subtract SD OD from Ag OD **Ag OD – SD OD = Net OD for Patients**	Divide the Net OD by the Cut-off OD **Net OD/CO OD = Index for Patients**	Interpretation
Non-detectable Control	0.008		0.02			
Cut-off	0.400		1.00			
Detectable Control	1.200		3.00			
Specimen A	0.860	0.020		0.840	2.10	POS
Specimen B	0.890	0.010		0.880	2.20	POS
Specimen C	0.760	**0.720**		0.040	**0.10**	NEG

* Blank OD is already subtracted from each result.

Discussion of Background Subtraction Example Calculations

Specimen A and B should be interpreted as IgM positive because the index after subtracting the background is still greater than 1.00. (the wells with no antigen was not reactive).

Specimen C demonstrates the importance of subtracting background. Specimen C was reactive even when Sample Diluent was added instead of DV antigen, indicating that antibodies in the sample were cross-linking the IgM Capture antibodies and the monoclonal conjugate. Specimen C should be considered IgM negative because the index value after subtracting background was 0.10, and 0.10 is less than 1.00 (thus in the negative interpretation zone).

INTERPRETATION OF TEST RESULTS

Report all patient results as index values relative to the Cut-off Calibrator: to calculate index values, divide specimen optical density (OD) values (corrected for blank readings) by the mean of the corrected Cut-off Calibrator absorbance values.

> 1.00	**Positive.** An index value of > 1.00 is presumptive for the presence of IgM antibodies to dengue virus.
< 1.00	**Negative.** An index value of < 1.00 indicates no IgM antibodies to dengue virus were detected.

LIMITATIONS

1. Dengue virus is a flavivirus. Other members of this group include Banzi virus, Bussuquara virus, Iiheus virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley Encephalitis virus, Rocio virus, Kunjin virus, Spondweni virus, Sepik virus, West Nile virus, yellow fever virus, Zika virus, Wesselsbron virus, Rio Bravo virus, Central European Encephalitis virus, Russian spring-summer virus, Kyasanur Forest Disease virus, Louping ill virus, Negishi virus, Omsk hemorragic fever virus and Powassan virus. While the majority of these viruses rarely cause disease in man, or are restricted to narrow geographical regions, cross reactions between members of Flaviviridae are common. Therefore, care should be taken in interpreting the results before a definitive diagnosis of dengue fever is made. These assays are not intended as a substitute for a physician's clinical impressions.
2. All results from this and other serologies must be correlated with clinical history, epidemiological data, and other data available to the attending physician in making the diagnosis of dengue virus infection.
3. If a negative test result is reported for a patient for whom dengue fever is strongly suspected on clinical or epidemiological grounds, a second serum sample should be drawn 7 to 10 days after the disease onset and retested for IgM class antibodies.

EXPECTED VALUES

In one unpublished study it was observed that at 7 days after onset more than 90% of confirmed dengue fever cases had detectable IgM by ELISA, and at 10 days after onset, 93% had detectable IgM by ELISA.[8] Serum from patients without signs, symptoms or previous history of dengue fever should give a negative test result with this procedure.

PERFORMANCE CHARACTERISTICS

For customers outside of the United States, the product performance characteristics are supplied as a separate sheet.

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This package insert is available in French, German, Italian, and Spanish at www.focusdx.com, and may be available in other languages from your local distributor.

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