Dengue Virus IgM µ-capture ELISA Kit (Human) (OKNX00132)

Instructions for use

For the qualitative detection of Anti-Dengue Virus µ-capture IgM in human serum.

This product is intended for research use only.

Lot to lot kit variations can occur. Use the kit manual which has been provided along with the kit packaging.

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1. Background

Principle

Aviva Systems Biology Dengue Virus IgM µ-capture ELISA Kit (Human) (OKNX00132) is based on standard reverse capture sandwich enzyme-linked immuno-sorbent assay technology. Dengue Virus µ-capture antigen has been pre-coated and blocked in a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and washed. An HRP conjugated detector antibody specific for Human IgM is added, incubated and followed by washing. An enzymatic reaction is produced through the addition of substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration is read by absorbance at 450 nm and is qualitatively proportional to the amount of sample anti-Dengue Virus µ-capture IgM captured the in well.

Background

Dengue fever, also known as breakbone fever, is an infectious tropical disease caused by the dengue virus and transmitted by mosquitoes.

Dengue fever virus (DENV) is a virus of the family Flaviviridae, genus Flavivirus and contains a single-stranded RNA genome with positive polarity. There are four serotypes of the virus, which are referred to as DENV-1, DENV-2, DENV-3 and DENV-4.

The geographical distribution is around the equator, particularly Latin America, Central Africa, India, Southeast Asia, Western Pacific and South of the USA.

Dengue viruses are transmitted to humans through the bites of infective female yellow fever mosquitoes (Stegomyia aegypti, formerly Aedes aegypti). The mosquitoes generally acquire the virus while feeding on the blood of an infected person. After virus incubation for eight to ten days, an infected mosquito is capable, during probing and blood feeding, of transmitting the virus for the rest of its life.

Yellow fever mosquitoes are well adapted to living in close proximity to humans, and to feeding off people rather than other vertebrates. They prefer to lay their eggs in artificial water containers, such as flower vases, uncovered barrels, buckets and discarded tires.

The incubation period ranges from 3-14 days, but most often it is 4-7 days. Typically, people infected with dengue virus are asymptomatic or only have symptoms of a common cold. The characteristic symptoms of dengue are sudden-onset fever (up to 40 °C) with intense headache (especially behind the eyes), and muscle and joint pain. In combination with a skin rash this symptoms are known as the „dengue triad“. This usually lasts 3-7 days. In some patients the disease proceeds to a critical phase. Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS) occur in less than 5 % of all cases of dengue. About 1-5 % of severe cases are fatal. In individual epidemics the case-fatality rate may reach up to 15 %.

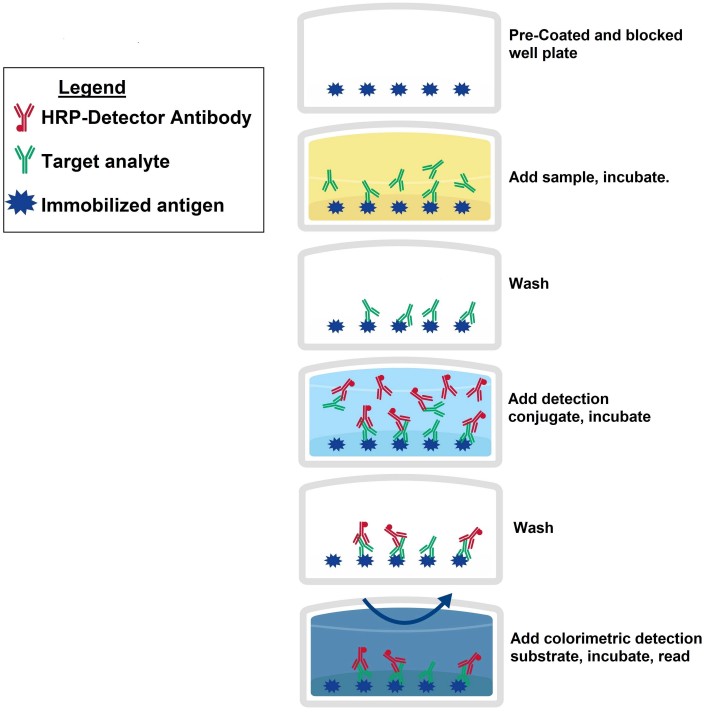
Infection with one serotype is believed to produce lifelong immunity to that serotype but only short term protection against the others. Secondary infection with a different serotype may result in severe clinical manifestations. There is no vaccination available.

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

The presence of virus or infection may be identified by

- Virus cultivation, RT-PCR
- Detection of NS1 antigen
- Serology: Detection of specific antibodies by ELISA

2. Assay Summary



3. Storage and Stability

• Upon receipt store kit at 4°C for 6 months.

4. Kit Components

•The following reagents are the provided contents of the kit.

Description		Quantity	Storage Conditions
Dengue Virus µ-capture IgM Microplate		96 Wells (12 x 8 Well strips)	4°C for 6 Months
Sample Diluent	White Cap	1 x 100 mL	
Stop Solution	Red Cap	1 x 15 mL	
20X Wash Solution	White Cap	1 x 50 mL	
Anti-Human IgM HRP Conjugate	Black Cap	1 x 20 mL	
TMB Substrate	Yellow Cap	1 x 15 mL	
Dengue Virus µ-capture IgM Cut-Off Control	Green Cap	1 x 3 mL	
Dengue Virus µ-capture IgM Positive Control	Red Cap	1 x 2 mL	
Dengue Virus µ-capture IgM Negative Control	Blue Cap	1 x 2 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

• The following reagents are provided at ready to use concentrations and require no preparation:

- Sample Diluent
- Stop Solution
- Anti-Human IgM HRP Conjugate
- TMB Substrate
- Dengue Virus µ-capture IgM Cut-Off Control
- Dengue Virus µ-capture IgM Positive Control
- Dengue Virus µ-capture IgM Negative Control

8.1 1X Wash Buffer

8.1.1 If crystals have formed in the 20X **Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.1.2 For 500 mL, add 25 mL contents of the 20X **Wash Buffer** bottle to 480 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.1.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.1.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.2 Microplate Preparation

• Micro-plates are provided ready to use and do not require rinsing or blocking.

• Unused well strips should be returned to the original packaging, sealed and stored at 4°C.

• Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C.Avoid repeated freeze-thaw cycles.

• Samples not indicated in the manual must be tested to determine if the kit is valid.

• Prepare samples as follows:

• Serum - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• Plasma - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

9.2 Sample Dilution (1:100)

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

• Dilute samples using **Sample Diluent**.

• Mix diluted samples gently and thoroughly.

• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

The suggested 100-fold dilution can be achieved by adding 10 µL sample to 990 µL of **Sample Diluent**.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for inter- and intra-assay reproducibility will be observed when incubation at 37°C as indicated in the procedure below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch. Allow additional wells to include the following (recommended in duplicate):

10.1.1 Substrate Blank

10.1.2 Negative Control

10.1.3 Cut-Off Control

10.1.4 Positive Control

10.2 Add 100 µL of the **1:100 diluted samples**, **Positive Control, Cut-Off Control** or **Negative Control** to test wells of the Dengue Virus µ-capture IgM Microplate. At least two replicates are recommended.

10.3 Cover the plate with the well plate lid and incubate at 37±1°C for 60±5 minutes.

10.4 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Wash plate 3 times with **1X Wash Buffer** as follows:

10.6.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.6.2 Allow to soak for ~ 5 seconds.

10.6.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.6.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6.5 Repeat steps 10.6.1 through 10.6.4 **two** more times.

10.7 Add 100 µL of the **1X Anti-Human IgM HRP-Conjugate** to all the wells.

10.8 Cover with the well-plate lid and incubate at room temperature for 30 minutes.

10.9 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.10 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.11 Repeat the wash in step 10.6.

10.13 Add 100 µL of **TMB Substrate** to each well and incubate at room temperature i**n the dark** for 15 minutes.

10.14 Add 100 µL of **Stop Solution** to each well in the same order as that of step 10.13.

10.15 Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.14. If wavelength correction is available, set to 620 nm.

11. Calculation of Results

A positive or negative Dengue Virus µ-capture IgM determination is derived by comparing the test samples to the **Positive** and **Negative Controls**.

11.1 Calculation of Results

- The **Cut-Off** is the mean 450 nm absorbance (A_{450}) value of the **Cut-Off Control** sample determinations.
- The Test Specimen measurement is the mean of the replicate A_{450} measurements.

11.2 Run Validation Criteria

For an assay to be considered valid, the following criteria must be met:

Substrate blank: Absorbance value < 0.100.

Dengue Virus µ-capture IgM Negative control: Absorbance value < 0.200 and < Cut-Off

Dengue Virus µ-capture IgM Cut-off Control: Absorbance value 0.150 – 1.30

Dengue Virus µ-capture IgM Positive control: Absorbance value > Cut-Off

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

11.3 Interpretation of Results

The criteria to establish the results of an assay run using mean absorbance values are as follows:

Test samples are considered POSITIVE if the absorbance value is higher than 10% over the Cut-Off.

Samples with an absorbance value of <10% above or below the Cut-Off should not be considered as clearly positive or negative (grey zone). It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample must be considered NEGATIVE.

Samples are considered NEGATIVE if the absorbance value is lower than 10% below the Cut-Off.

12 Typical Expected Data

12.1 Precision

Replicate samples were measured to assess inter- and intra-assay reproducibility.

Metric	Sample Type	n=	Mean	CV%
Inter-Assay	Serum #2	12	20.02	4.8
Inter-Assay	Serum #4	12	8.96	6.2
Inter-Assay	Serum #5	12	5.32	5.8
Intra-Assay	Serum #1	23	0.530	3.2
Intra-Assay	Serum #2	24	1.019	2.4
Intra-Assay	Serum #3	24	0.986	2.8

12.2 Specificity

Specificity is determined as the probability of the assay indicating a negative score in samples absent of the specific analyte: >97.5%

12.3 Sensitivity

Sensitivity is determined as the probability of the assay indicating a positive score in samples with the specific analyte present: >98%

12.4 Interference Factors

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

12.5 Cross-Reactivity

Disease Type	Total Specimens	Positive Result
Anti-nuclear antibody	4	0/4
Chikungunya Virus	6	0/6
CMV	6	0/6
Epstein-Barr Virus	4	0/4
FSME	2	0/2
Leptospira	4	0/4
Malaria	4	0/4
Rheumatoid Factor	24	2/24
Rubella Virus	2	0/2
West Nile Virus	15	2/15
Total	**71**	**4/71**

13 Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

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