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ab108728 – Anti-Dengue virus IgG Human ELISA Kit

Instructions for Use

For the qualitative measurement of IgG class antibodies against Dengue virus in Human serum and plasma (heparin and citrate)

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's anti-Dengue virus IgG Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate qualitative measurement of IgG class antibodies against Dengue virus in Human serum and plasma.

A 96-well plate has been precoated with Dengue virus antigens to bind cognate antibodies. Controls or test samples are added to the wells and incubated. Following washing, a horseradish peroxidase (HRP) labelled anti-Human IgG conjugate is added to the wells, which binds to the immobilized Dengue virus-specific antibodies. TMB is then catalyzed by the HRP to produce a blue color product that changes to yellow after adding an acidic stop solution. The density of yellow coloration is directly proportional to the amount of Dengue virus IgG sample captured in plate.

Dengue virus is a single-stranded RNA virus of about 50 nm in diameter belonging to the genus Flavivirus. Dengue and dengue hemorrhagic fever are caused by one of four closely related, but antigenically distinct, virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4). Infection with one of these serotypes does not provide crossprotective immunity, so persons living in a dengue-endemic area can have four dengue infections during their lifetimes. The viruses are transmitted by Aedes aegypti, a domestic, day-biting mosquito that mainly feeds on Humans. Infection with dengue viruses produces a spectrum of clinical illness ranging from a nonspecific viral syndrome to severe and fatal hemorrhagic disease. It is primarily a disease of the tropics; its global distribution is comparable to that of malaria, and an estimated 2.5 billion people live in areas at risk for epidemic transmission. Globally, there are an estimated 50 to 100 million cases of dengue fever and several hundred thousand cases of dengue hemorrhagic fever.

- The case-fatality rate of DHF in most countries is about 5%; most fatal cases are among children and young adults.
- Important risk factors for DHF include the strain and serotype of the infecting virus, as well as the age, immune status, and genetic predisposition of the patient.
- Risk groups: residents of or visitors to tropical urban areas.

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

The presence of viral infection may be identified by

- Serology: Detection of antibodies by ELISA

Infection produces lifelong immunity, but the antigenically distinct serotypes do not provide cross-protective immunity, so a person can theoretically experience four dengue infections; a dengue vaccine is not available.

2. Protocol Summary

Prepare all reagents, samples and controls as instructed.

Add standard or sample to appropriate wells.

Incubate at 37°C.

Wash each well and add prepared labeled HRP-Conjugate. Incubate at room temperature

After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures

- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious

- Use only clean pipette tips, dispensers, and lab ware.

- Do not interchange screw caps of reagent vials to avoid cross-contamination

- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination

- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

6. Materials Supplied

Item	Amount	Storage Condition (Before Preparation)
Dengue virus (IgG) Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
IgG Sample Diluent***	100 mL	2-8°C
Stop Solution	15 mL	2-8°C
20X Washing Solution*	50 mL	2-8°C
Dengue virus anti-IgG HRP Conjugate**	20 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
Dengue virus IgG Positive Control***	2 mL	2-8°C
Dengue virus IgG Cut-off Control***	3 mL	2-8°C
Dengue virus IgG Negative Control***	2 mL	2-8°C

* Contains 0.1 % Bronidox L after dilution

** Contains 0.2 % Bronidox L

*** Contains 0.1 % Kathon

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450nm or 620 nm
- Incubator at 37°C
- Multi and single channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

8. Technical Hints

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions

9. Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.

9.1 1X Washing solution

Prepare 1X Washing Solution by diluting 20X Washing Solution with deionized water. To make 200 mL 1X Washing Solution combine 10 mL 20X Washing Solution with 190 mL deionized water. Mix thoroughly and gently.

All other solutions supplied are ready to use.

10. Sample Collection and Storage

Use Human serum or plasma (heparin or citrate) samples with this assay. If the assay is performed within 5 days of sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20 to -80°C). If samples are stored frozen, mix thawed samples well before testing.

Avoid repeated freezing and thawing.

Heat inactivation of samples is not recommended

11. Sample Preparation

Before assaying, all samples should be diluted 1:100 with IgG Sample Diluent. Add 10 µL sample to 1 mL IgG Sample Diluent to obtain a 1:100 dilution. Mix gently and thoroughly.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

Refer to Dilution Guidelines for further instruction.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

13. Assay Procedure

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Reliability of results depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **All controls (Dengue virus IgG Positive, Dengue virus IgG Negative and Dengue virus IgG Cut-off) must be included with each assay performed to determine test results**
- **Assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3. Add 100 µL of controls and diluted samples into appropriate wells. Leave one well for substrate blank.

13.4. Cover wells with the foil supplied in the kit and incubate for 1 hour at 37°C.

13.5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

Note: Complete removal of liquid at each step is essential for good assay performance.

13.6. Add 100 µL Dengue virus anti-IgG HRP Conjugate into all wells except for the blank well. Cover with foil.

13.7. Incubate for 30 minutes at room temperature. Do not expose to direct sunlight.

13.8. Repeat step 13.5.

13.9. Add 100 µL TMB Substrate Solution into all wells

13.10. Incubate for exactly 15 minutes at room temperature in the dark.

13.11. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.

Note: Any blue color developed during the incubation turns into yellow.

13.12. Highly positive samples can cause dark precipitates of the chromogen. These precipitates have an influence when reading the optical density. Predilution of the sample with PBS for example 1:1 is recommended. Then dilute the sample 1:100 with IgG Sample Diluent and multiply the results in Standard Units by 2 (See Section 14. Calculations.)

13.13. Measure the absorbance of the specimen at 450 nm within 30 minutes of addition of the Stop Solution.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

14. Calculations

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank:** Absorbance value **< 0.100**
- **Negative control:** Absorbance value **< 0.200 and < cut-off**
- **Cut-off control:** Absorbance value **0.150 – 1.300**
- **Positive control:** Absorbance value **> cut-off**

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

Calculation of Results

Calculate the mean background subtracted absorbances for each sample and compare to mean Cut-off control value.

The Cut-off control value is the mean absorbance value of the Cut-off control wells.

Example: Absorbance value Cut-off control Well 1 = 0.156

Absorbance value Cut-off control Well 2 = 0.168

Mean Cut Off value: (0.156 + 0.168)/2 = 0.162

Interpretation of Results

Samples are considered to give a positive signal if the absorbance value is greater than 10% over the cut-off value.

Samples with an absorbance value of less than 10% above or below the Cut-off control value should be considered as inconclusive (grey zone) i.e. neither positive or negative. It is recommended to repeat the assay using fresh samples. If results of the second test are again less than 10% above or below the Cut-off control value the sample has to be considered negative.

Samples are considered negative if the absorbance value is lower than 10% below the cut-off.

Results in Standard Units

$$\frac{\text{Patient (mean) absorbance value x 10}}{\text{Cut-off}} = \text{Standard Units}$$

Example: $\frac{1.786 \times 10}{0.38} = 47$ *Standard Units*

Cut-off:	10	Standard Units
Grey zone:	9-11	Standard Units
Negative:	<9	Standard Units
Positive:	>11	Standard Units

15. Typical Sample Values

Precision -

Positive Serum	Intra-Assay	Inter-Assay
n=	8	8
Mean	0.98	0.47
%CV	4.34	6.76

16. Assay Specificity

SPECIFICITY -

The specificity is 93 % and is defined as the probability of the assay scoring negative in the absence of the specific analyte.

SENSITIVITY -

The sensitivity is > 90 % and is defined as the probability of the assay scoring positive in the presence of the specific analyte.

17. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.2 mg/mL bilirubin.

18. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

Problem	Cause	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Try overnight incubation at 4 °C
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.

	Contaminated wash buffer	Prepare fresh wash buffer
	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
High Background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

19. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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