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# ab177835 Anti-Chikungunya Virus IgG Human ELISA Kit

For the quantitative measurement of human albumin in plasma, serum, urine and cell culture supernatants.

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

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# 1. Overview

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

A 96-well plate has been precoated with anti-Human antibodies to bind to corresponding antibodies of the sample. Controls or test samples are added to the wells and incubated. Following washing, the Chikungunya Virus antigen is added to the wells and incubated. The wells are then washed as before and the biotinylated Chikungunya Virus antibodies are added to each well followed by incubation. After a last wash step, the streptavidin peroxidase (SP) conjugate which binds to the biotinylated Chikungunya Virus-specific antibodies is added to the wells. TMB is then catalyzed by the SP 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 Chikungunya Virus IgG sample captured in plate.

Chikungunya virus is an arthropod borne virus of the genus Alphavirus (family Togaviridae). The Alphavirus genus contains at least 24 distinct species. These are lipid-enveloped virions with a diameter of 50 to 60 nm. Alphavirus is transmitted by the bite of an infected mosquito. After an incubation period of 1 to 12 days the Chikungunya fever develops. Chikungunya fever (Chikungunya means "that which bends up", in reference to the crippling manifestations of the disease) is an acute viral infection characterized by a rapid transition from a state of good health to illness that includes severe arthralgia and fever. Temperature rises abruptly to as high as 40°C and is often accompanied by shaking chills. After a few days, fever may come and go, giving rise to a "saddleback" fever curve. Arthralgia is polyarticular, favoring the small joints and sites of previous injuries, and is most intense on arising. Patients typically avoid movement as much as possible. Joints may swell without significant fluid accumulations. These symptoms may last from 1 week to several months and are accompanied by myalgia. A rash characteristically appears on the first day of illness, but onset may be delayed. It usually arises as a flush over the face and neck, which evolves to a maculopapular or macular form that may be pruritic. The latter lesions appear on the trunk, limbs, face, plams and soles, in that

order of frequency. Petechial skin lesions have also been noted. Headache, photophobia, retro-orbitral pain, sore throat with objective signs of pharyngitis, nausea and vomiting also occur in this setting. Occasionally, however, persistent arthralgia and polyarthritis (lasting months or even years) do occur, sometimes involving joint destruction. Even rarer sequelae include encephalitis and meningoencephalitis with high lethality rates. The virus has major importance in Africa and Asia. From 20% to more than 90% of the population of tropical and subtropical show serologic evidence of infection. The increasing prevalence of Aedes mosquitoes in North Africa and South America gives the possibility of emerging epidemics. Chikungunya virus infections can occur in central Europe due to travellers returning from tropical and subtropical countries.

| Species | Diseases | Symptoms | Mechanism of infection |
|---|---|---|---|
| Chikungunya virus (Alphavirus) | Chikungunya fever | Fever<br>Exanthema<br>Joint pain<br>Persistent arthralgia and polyarthritis, sometimes involving joint destruction.<br>Even rarer encephalitis and meningoencephalitis | Transmission by bloodsucking mosquitoes<br>Aedes albopictus (Africa)<br>Aedes aegypti (Africa, Asia) |

The presence of viral infection may be identified by:

- Serology: Detection of antibodies by IF, ELISA

## 2. Protocol Summary

Prepare all reagents, samples, controls and standards as instructed.

⇩

Add samples, standards and controls to wells used.

⇩

After washing, add virus antigen to each well and incubate.

⇩

After washing, add labeled biotinylated antibody to each well and incubate.

⇩

After washing, add labeled SP-Conjugate to each well and incubate.

⇩

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

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Anti-Human (IgG) Coated Microplate (12 x 8 wells) | 96 wells | 4°C |
| IgG Sample Diluent*** | 100 mL | 4°C |
| Stop Solution | 15 mL | 4°C |
| 20X Washing Solution* | 50 mL | 4°C |
| Chikungunya Virus antigen Solution 1 (Lyophilized)**** | 6 vials | 4°C |
| Chikungunya Virus biotinylated antibody Solution 2**** | 6 mL | 4°C |
| Streptavidin Conjugate** | 6 mL | 4°C |
| TMB Substrate Solution | 15 mL | 4°C |
| Chikungunya Virus IgG Positive Control**** | 1.5 mL | 4°C |
| Chikungunya Virus IgG Cut-off Control**** | 2 mL | 4°C |
| Chikungunya Virus IgG Negative Control*** | 1.5 mL | 4°C |

* Contains 0.1 % Bronidox L after dilution
** Contains 0.2 % Bronidox L
*** Contains 0.1 % Kathon
**** Contains 0.02 % Kathon and 0.02% Bronidox L after reconstitution

## 7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm 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.
- Prepare only as much reagent as is needed on the day of the experiment.

## 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.

## 9.2 1X Chikungunya Virus Solution 1

Add 1 mL of 1X Washing Solution to each vial. Incubate for 15 minutes at room temperature with gentle mixing. The reconstituted solution is stable for 24 hours at 4°C.

- All other solutions are supplied ready to use.

## 10. Sample Preparation

Use Human serum or plasma (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.

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.

*Refer to Dilution Guidelines for further instruction.*

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

## 11. 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 well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition.
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

## 12. 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 (Chikungunya Virus IgG Positive, Chikungunya Virus IgG Negative and Chikungunya Virus IgG Cut-off) must be included with each assay performed to determine test results
- Assay all standards, controls and samples in duplicate

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

**12.2** 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.

**12.3** Add 50 µL of controls or diluted sample into appropriate wells. Leave one well for substrate blank.

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

**12.5** 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 at least greater than 5 seconds. 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.

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

**12.6** Add 50 µL 1X Chikungunya Virus Solution 1 into all wells except for the blank well. Cover with foil.

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

**12.8** Repeat washing steps described in step 12.5.

12.9 Add 50 µL Chikungunya Virus Solution 2 into all wells except for the blank well. Cover with foil. Incubate for 30 minutes at room temperature. Do not expose to direct sunlight.

12.10 Repeat washing steps described in step 12.5.

12.11 Add 50 µL SP Conjugate into all wells except for the blank well. Cover with foil. Incubate for 30 minutes at room temperature. Do not expose to direct sunlight.

12.12 Repeat washing steps described in step 12.5.

12.13 Add 100 µL TMB Substrate Solution into all wells

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

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

ΔNote: Any blue color developed during the incubation turns into yellow. 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 13, Calculations).

12.16 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.

## 13. 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 **< 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: $\frac{1.786 \times 10}{0.38} = 47 \text{ Standard Units}$

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

## 14. Typical Sample Values

### SPECIFICITY –

The specificity is > 90 % 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.

### PRECISION –

| Low positive serum | Intra-Assay | Inter-Assay |
|---|---|---|
| n= | 23 | 3 |
| Mean | 0.39 | 0.39 |
| %CV | 9.4 | 10.9 |

| High Positive serum | Intra-Assay | Inter-Assay |
|---|---|---|
| n= | 24 | 3 |
| Mean | 1.44 | 1.41 |
| %CV | 2.7 | 3.7 |

## 15. Assay Specificity

### CROSS REACTIVITY –

Samples with IgG antibodies against Dengue virus, Tick born encephalitis, CMV, EBV and Helicobacter pylori showed no cross reactivity. Cross reactivity with antibodies against O´Nyong Nyong virus is not excluded.

### INTERFERENCES –

No interferences were observed when adding trigycerides, bilirubin and haemoglobin in an excess to the sample.

Please contact our Technical Support team for more information.

## 16. Troubleshooting

| Problem | Cause | Solution |
|---|---|---|
| Low signal | Incubation time to short | Try overnight incubation at 4 °C |
| | 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 |
| | Sample prepared incorrectly | Ensure proper sample preparation/dilution |
| Large CV | 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 |
| | Inconsistent sample preparation or storage | Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles) |

| Problem | Cause | Solution |
|---|---|---|
| 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 | Improper storage of ELISA kit | Store all reagents as recommended. Please note all reagents may not have identical storage requirements. |
| | Using incompatible sample type (e.g. Serum vs. cell extract) | Detection may be reduced or absent in untested sample types |

# 17. Notes

## Technical Support



**For all technical or commercial enquiries please go to:**

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)