INTENDED USE

The **Human Anti-Chikungunya Virus (CHIKV) E1 IgG** ELISA Kit detects and quantifies anti-CHIKV E1 IgG in human serum or plasma of exposed or immunized animals. This immunoassay is suitable for:

- Determining immune status relative to non-immune controls;
- Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and/or standardizing vaccine batches and protocols.

This kit is for research use only (RUO), not for diagnosis or therapeutic purposes.

INTRODUCTION

Chikungunya virus (CHIKV) is an arthritogenic arbovirus belonging to the alphavirus genus of Togaviridae, transmitted to humans by infected female Aedes arthropods. CHIKV can cause acute infections with fever and severe joint pain; also chronic rheumatism. So far, CHIKV has been identified in over 60 countries in Europe, the Americas, Asia and Africa.

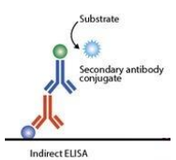
CHIKV is an enveloped, single-stranded positive-sense RNA virus, with a genome of about 11.7 kb including 5' and 3' ORFs. The 5' ORF is translated from genomic RNA by a cap-dependent mechanism resulting in the formation of 5 structural (envelope proteins E1-E3 forming trimeric spikes on the virions' surface, capsid and 6K/TF) and 4 non-structural proteins (nsP1-4).

While glycoprotein (GP) E1 is responsible for fusion within endosomes of target cells and nucleocapsid release, GP E2 interacts with cellular receptors for cell entry. The small GP E3 mediates pH-protection during virus biogenesis and prevents E1 from premature fusion. The capsid protein of alphavirus serves as a serine protease for self-cleavage, is necessary for the interaction with viral spike proteins during virion formation and serves a major function in nucleocapsid formation. The 6K/TF protein is essential for formation and budding of new virions.

Two-thirds of CHIK virus' RNA encodes for non-structural polyprotein precursors nsP1-4 serving RNA helicase, nucleoside triphosphatase and RNA dependent 5' triphosphatase enzymatic activities.

Vaccine development activities have focused primarily on the structural E1, E2, E3 glycoproteins; efforts have also included targeting the capsid, 6K and some non-structural proteins.

PRINCIPLE OF THE TEST



The **Human Anti- Chikungunya Virus E1 IgG** ELISA kit is based on the binding of human anti- CHIKV E1 IgG in samples to CHIKV E1 immobilized on the microwells, and anti- CHIKV E1 IgG antibody is detected by anti-Human IgG specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti- CHIKV E1 IgG present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of human IgG antibody in samples is calculated relative to anti- CHIKV E1 calibrators.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8° C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at 4° C for long term and RT for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample/Conjugate Diluent** and store at 2-8° C until the kit lot expires or is used up.
Anti-Human IgG-HRP Conjugate Concentrate (100x) Part: H-HuG.2a11, 0.15ml	Peroxidase conjugated anti-human IgG in buffer with detergents and antimicrobial. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8° C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
CHIKV E1 Coated Strip Plate	530-421	8-well strips (12)	Coated with Chikungunya E1 virus proteins; post-coated with stabilizers.
Anti- CHIKV E1 Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	530-422B 530-422C 530-422D 530-422E	0.65ml 0.65ml 0.65ml 0.65ml	Four (4) vials, each containing anti-CHIKV E1 antibodies; in buffer with antimicrobial.
Anti- CHIKV E1 Positive Control	530-422PC	0.65ml	Anti- CHIKV E1 diluted in buffer with protein, detergents and antimicrobial. [Value range on label]
Low NSB Sample Diluent Reduces non-specific binding	TBTm **Not** for HRP Conjugate dilution	30 ml	Buffer with protein, detergents and antimicrobial. Use as is for sample dilution. See **Assay Design**, page 3.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- ELISA reader at 450 nm and ELISA plate washer

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Antibody Stability & Dilution

Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 5 times the initial dilution and performed the same week as the assay.

Example: Initial (1/5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1/50): **10**ul initial (1/5) + **90**ul LNSD (1/50)

Assay Design

Review Interpretation of Results (p. 5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **1 U/ml Calibrator**. This is usually 1:100 or greater dilution for human serum with normal levels of IgG and IgM.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run the **Anti-CHIKV E1 Positive Control**; the value range is on the label.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Plate Set-up

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

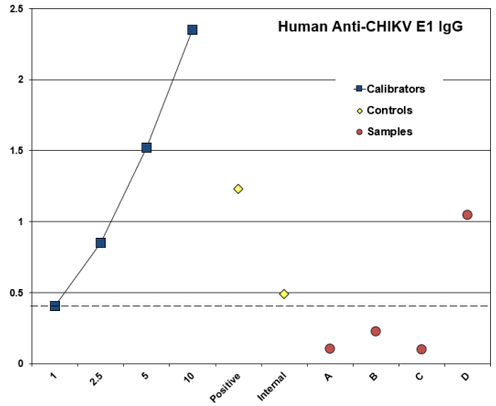
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti- CHIKV E1 IgG, from either natural infection or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of antiserum from CHIKV E1 virus immunization, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – serum showing reactivity to CHIKV E1 virus; the value range is on the label. This Control may be used to gauge precision and to normalize between-assay variation.

Internal Control – a true positive from an immune human that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (1:100) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

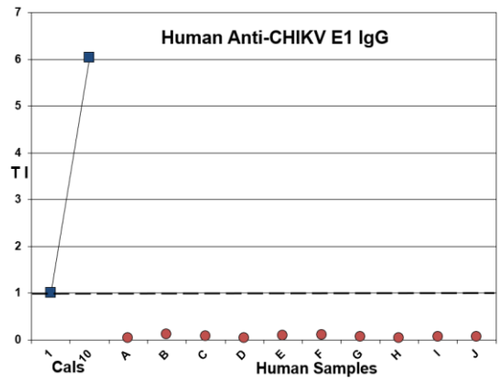
The 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Example:

Human Serum IgG
A panel of sera from non-immunized individuals was tested for anti- Chikungunya Virus E1 IgG (1:100 dilution in Low NSB Sample Diluent). **Threshold Index** was calculated using the **1** U/ml **Calibrator**.



Results

Anti-CHIKV E1 virus IgG:
All ten of the human sera were negative (clearly below 1.0 TI) at 1:100 dilution.

Notes:

1. **Positives** may be due to prior encounter with the virus, from exposure to an antigen with common epitopes, or from immunization.
2. When the **Positive Index** is **above 5.0,** using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
3. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:200) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:50) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index (**see below) or use an **Internal Control** (Page 5).

B. Positive Index
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Method C. Titers from Sample Dilution Curves
The titer of elevated antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Calibrator values can be used to normalize inter-assay values.

Calculations
1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index

= IgG Antibody Activity Units

PRODUCT SPECIFICATIONS

Specificity
Recombinant wild-type Chikungunya Virus E1 Envelope protein ectodomain is used to coat the microwells; thus the assay is specific for antibodies directed to the CHIKV E1. The Anti-Human IgG-HRP conjugate reacts specifically with human IgG class antibodies; IgA, IgM and IgE antibody would not be measured above background signals.

Assay Sensitivity
The CHIKV E1 virus coating level, HRP conjugate concentration and Low NSB Sample Diluent are optimized to differentiate anti-CHIKV E1 IgG from background (non-antibody) signal with human serum samples diluted 1:100.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website.

Instruction Manual No. M-530-420-CEG

Human Anti-Chikungunya Virus E1 IgG ELISA Kit

Cat. #530-420-CEG, 96 tests

For Quantitation of Anti-Chikungunya Virus E1 IgG in Serum, Plasma, or other Biological Fluids

For research use only, not for diagnostic or therapeutic use.

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 W • San Antonio • Texas 78249 • USA

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777
www.4adi.com
service@4adi.com

ELISA Kit Components		Amount	Part #
CHIKV E1 Coated Strip Plate		8-well strips (12)	530-421
Anti-CHIKV E1 Positive Control		0.65 ml	530-422PC
Anti-CHIKV E1 Calibrator	1 U/ml	0.65 ml	530-422B
Anti-CHIKV E1 Calibrator	2.5 U/ml	0.65 ml	530-422C
Anti-CHIKV E1 Calibrator	5 U/ml	0.65 ml	530-422D
Anti-CHIKV E1 Calibrator	10 U/ml	0.65 ml	530-422E
Anti-Human IgG HRP Conjugate (100X)		0.15 ml	H-HuG.2a11
Sample Diluent (20x)		10 ml	SD20T
Low NSB Sample Diluent		30 ml	TBTm
Wash Solution Concentrate (100X)		10 ml	WB-100
TMB Substrate		12 ml	80091
Stop Solution		12 ml	80101
Product Manual		1 ea	M-530-420-CEG