Chikungunya IgM micro-capture ELISA

Enzyme immunoassay for the qualitative determination of IgM-class antibodies against Chikungunya virus in human serum or plasma.

REF RE58841

Σ 96

2-8°C

U.S.: For research use only. Not for use in diagnostic procedures.

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1. INTENDED USE

The Chikungunya IgM micro capture ELISA is intended for the qualitative determination of IgM class antibodies against Chikungunya Virus in human serum or plasma (citrate, heparin). For research use only. Not for use in diagnostic procedures.

2. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific IgM-class antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) μ-capture technique. Microplates are coated with anti-human IgM antibodies to bind the corresponding antibodies of the sample. After washing the wells to remove all unbound sample material, antigen is added. This antigen binds to the captured specific IgM antibodies. After a further washing step biotinylated antibody is pipetted into the wells. After washing horseradish peroxidase (HRP) labelled streptavidin is added that binds to the captured specific immune complex. After a further washing step the immune complexes are visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific IgM antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

3. MATERIALS

3.1. Reagents supplied

- **MTP Chikungunya Virus Coated Microplate (IgM):** 12 break-apart 8-well snap-off strips coated with anti-human IgM; in resealable aluminium foil.
- **SAMPLEDIL Sample Diluent:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
- **STOP Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **WASHBUF CONC Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **ANTIGEN LYO Chikungunya Virus antigen, lyophilized:** 6 bottles containing lyophilized Chikungunya virus antigen solution; red cap.
- **ANTIBODY SOL Chikungunya Virus antibody solution:** 1 bottle containing 6 mL of biotinylated Chikungunya virus antibody, ready to use; coloured blue; white cap.
- **ENZCONJ Streptavidin conjugate:** 1 bottle containing 6 mL Streptavidin conjugated with peroxidase, ready to use; coloured red; black cap.
- **TMB SUBS TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap; < 5 % NMP.
- **CONTROL + Chikungunya Virus IgM Positive Control:** 1 vial containing 1,5 mL control (human serum or plasma); coloured yellow; ready to use; red cap.
- **CONTROL CO Chikungunya Virus IgM Cut-off Control:** 1 vial containing 2 mL control (human serum or plasma); coloured yellow; ready to use; green cap.
- **CONTROL - Chikungunya Virus IgM Negative Control:** 1 vial containing 1,5 mL control (human serum or plasma); coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

3.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)

3.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

4. STABILITY AND STORAGE

Store the kit at 2 - 8°C. The opened reagents are stable up to the expiry date stated on the label when stored at 2 - 8°C.

5. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20 - 25°C) and mix them before starting the test run!

5.1. Coated Microplate

The break-apart snap-off strips are coated with anti-human IgM. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 - 8°C.

5.2. Chikungunya Virus Antigen

The bottles contain lyophilized Chikungunya virus antigen solution. The content of each vial has to be resolved in 1 mL diluted Washing Buffer by turning it slowly (no vortex) and 15 min incubation at room temperature (20 - 25°C). The reconstituted solution is stable for 1 day at 2 - 8°C.

5.3. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20 - 25°C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

5.4. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2 - 8°C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

6. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2 - 8°C; otherwise they should be aliquoted and stored deep-frozen (-70 to -20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Heat inactivation of samples is not recommended.

6.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with Sample Diluent. Dispense 10 µL sample and 1 mL Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

7. ASSAY PROCEDURE

7.1. Test Preparation

Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the Plate layout. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 ºC.

1. Dispense 50 µL standards/controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.

5. Dispense 50 µL reconstituted Chikungunya virus Antigen into all wells except for the Substrate Blank well A1.

6. **Incubate for 30 min at room temperature (20 - 25°C).**

7. Repeat step 4.

8. Dispense 50 µL Chikungunya virus Antibody Solution into all wells except for the Blank well A1.

9. **Incubate for 30 min at room temperature (20 - 25°C).**


11. Dispense 50 µL Streptavidin peroxidase conjugate into all wells except for the Blank well A1.

12. **Incubate for 30 min at room temperature (20 - 25°C).** Do not expose to direct sunlight.


14. Dispense 100 µL TMB solution into all wells

15. **Incubate for exact 15 min. at room temperature (20 - 25°C) in the dark.** A blue colour occurs due to an enzymatic reaction.

16. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.

17. Measure the absorbance at 450/620nm within 30 min after addition of the Stop Solution.

7.2. Measurement

Adapt the ELISA microwell plate reader to zero using the Substrate Blank.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

**Measure the absorbance** of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the Plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

8. RESULTS

8.1. Run Validation Criteria

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.

8.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off control 0.44 + absorbance value Cut-off control 0.42 =0.86 / 2 = 0.43

Cut-off = 0.43

9.2.1 Results in Units [U]

\[
\text{Sample (mean) absorbance value} \times 10 \quad \text{Cut-off} = \text{[Units = U]}
\]

Example: \[\frac{1.591 \times 10}{0.43} = 37 \text{ U}\]
9. SPECIFIC PERFORMANCE CHARACTERISTICS

9.1. Precision

<table>
<thead>
<tr>
<th>Intraassay</th>
<th>n</th>
<th>Mean (OD)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>24</td>
<td>0.287</td>
<td>8.53</td>
</tr>
<tr>
<td>#2</td>
<td>24</td>
<td>0.769</td>
<td>5.82</td>
</tr>
<tr>
<td>#3</td>
<td>24</td>
<td>0.618</td>
<td>5.50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interassay</th>
<th>n</th>
<th>Mean (U)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>12</td>
<td>32.42</td>
<td>4.88</td>
</tr>
<tr>
<td>#2</td>
<td>12</td>
<td>26.96</td>
<td>5.82</td>
</tr>
<tr>
<td>#3</td>
<td>12</td>
<td>5.95</td>
<td>12.84</td>
</tr>
</tbody>
</table>

9.2. Interferences

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

9.3. Cross Reactivity

Cross reactivity with antibodies against Borrelia, CMV and Toxoplasma cannot be excluded. Interference with polyclonal stimulation of EBV infections is likely. In the presence of infectious Mononucleosis (Pfeiffer’s Disease, EBV infection) polyclonal stimulation of B lymphocytes can occur. This may result in non-specific reactions in the detection of antibodies of the IgM class. Therefore it is recommended to exclude an EBV infection by additional testing.

Cross reactivity with antibodies against other alpha viruses cannot be excluded.

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

11. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the samples.
- Only for research use. Not for use in diagnostic procedures.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- 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.
- **The Chikungunya virus antigens are inactivated. All materials should still be regarded and handled as potentially infectious. Wear gloves while performing the test. We recommend using the antigen under BSL2 cabinet (clean bench).**
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- 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 standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.
11.1. Disposal Considerations
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

12. ORDERING INFORMATION
Prod. No.: RE58841 Chikungunya IgM micro-capture ELISA (96 Determinations)
BIBLIOGRAPHY


Hochedez, Patrick; Jaureguiberry, Stephane; Debruyne, Monique; Bossi, Philippe; Hausfater, Pierre; Brucker, Gilles et al. (2006): Chikungunya infection in travelers. In Emerging infectious diseases 12 (10), pp. 1565–1567. DOI: 10.3201/eid1210.060495.


ABBREVIATIONS

| NMP | N-Methyl-2-pyrrolidone |
SUMMARY OF TEST PROCEDURE

SCHEME OF THE ASSAY
Chikungunya IgM micro-capture ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls on the Plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

<table>
<thead>
<tr>
<th>Substrate Blank (A1)</th>
<th>Negative control</th>
<th>Cut-off control</th>
<th>Positive control</th>
<th>Sample (diluted 1+100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>-</td>
<td>50µL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cut-off control</td>
<td>-</td>
<td>-</td>
<td>50µL</td>
<td>-</td>
</tr>
<tr>
<td>Positive control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50µL</td>
</tr>
<tr>
<td>Sample (diluted 1+100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50µL</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit

**Incubate for 1 h at 37°C**
Wash each well three times with 300µL of Washing Buffer

<table>
<thead>
<tr>
<th>Reconstituted Antigen</th>
<th>-</th>
<th>50µL</th>
<th>50µL</th>
<th>50µL</th>
<th>50µL</th>
</tr>
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</table>

**Incubate for 30 min at room temperature (20 - 25°C)**
Wash each well three times with 300µL of Washing Buffer

<table>
<thead>
<tr>
<th>Antibody Solution</th>
<th>-</th>
<th>50µL</th>
<th>50µL</th>
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</thead>
</table>

**Incubate for 30 min at room temperature (20 - 25°C)**
Wash each well three times with 300µL of Washing Buffer

<table>
<thead>
<tr>
<th>Streptavidin conjugate</th>
<th>-</th>
<th>50µL</th>
<th>50µL</th>
<th>50µL</th>
<th>50µL</th>
</tr>
</thead>
</table>

**Incubate for 30 min at room temperature (20 - 25°C)**
Do not expose to direct sunlight
Wash each well three times with 300µL of Washing Buffer

<table>
<thead>
<tr>
<th>TMB Substrate solution</th>
<th>100µL</th>
<th>100µL</th>
<th>100µL</th>
<th>100µL</th>
<th>100µL</th>
</tr>
</thead>
</table>

**Incubate for exact 15 min at room temperature (20 - 25°C) in the dark**
Stop solution 100µL 100µL 100µL 100µL 100µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)
<table>
<thead>
<tr>
<th>Symbols / Symbole / Symbôles / Símbolos / Σύμβολα</th>
</tr>
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<tbody>
<tr>
<td>REF</td>
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<tr>
<td>LOT</td>
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Symbols of the kit components see MATERIALS SUPPLIED. 
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. 
Voir MATERIEL FOURNIS pour les symbôles des composants du kit. 
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. 
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. 
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. 
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER’S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.