Product information

Useŕs Manual

Chikungunya Virus IgM µ-capture ELISA

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

REF **IB79813**

Σ **96**

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

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1. INTENDED USE

The Chikungunya IgM µ-capture ELISA is intended for the determination of IgM class antibodies to Chikungunya virus in human serum and 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 spe-cific 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 prod-uct 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

- **SORB MT Chikungunya Virus Coated Microplate (IgM):** 12 break-apart 8-well snap-off strips coated with anti-human IgM; in resealable aluminium foil.
- **SAM DIL 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 SOLN Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **WASH SOLN 20x 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.
- **Ag Chikungunya Virus antigen, lyophilized**: 6 bottles containing lyophilized Chikungunya virus antigen solution; red cap.
- **Ab SOLN Chikungunya Virus antibody solution**: 1 bottle containing 6 ml of biotinylated Chikungunya virus antibody, ready to use; coloured blue; white cap.
- **ENZ CONJ Streptavidin conjugate**: 1 bottle containing 6 ml Streptavidin conjugated with peroxidase, ready to use; coloured red; black cap.
- **SUB TMB TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap; < 5 % NMP.
- **CAL C Chikungunya Virus IgM Positive Control:** 1 vial containing 1,5 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **CAL B Chikungunya Virus IgM Cut-off Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **CAL A 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)
- 1 Plate layout

3.3. Materials and Equipment needed

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

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, samples and controls to room temperature (20…25°C) 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…-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 supplied in the kit. 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 °C.

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).**
10. Repeat step 4.
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.
13. Repeat step 4.
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

Adjust 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. Assay Validation Criteria

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

- **Substrate blank** in A1: Absorbance value **< 0.100**
- **Negative control** in B1: Absorbance value **< cut-off**
- **Cut-off control** in C1 and D1: Absorbance value **0.150 - 1.300**
- **Positive control** in E1: Absorbance value > cut-off

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

8.2. 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. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.
For further information about the specific performance characteristics please contact IBL-America.

9.1. Precision

Intraassay	n	Mean (OD)	CV (%)
#1	24	0.287	8.53
#2	24	0.769	5.82
#3	24	0.618	5.50

Interassay	n	Mean (U)	CV (%)
#1	12	32.42	4.88
#2	12	26.96	5.82
#3	12	5.95	12.84

9.2. Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 100.0% (95% confidence interval: 95.01% - 100.0%).

9.3. Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100.0% (95% confidence interval: 96.19% - 100.0%).

9.4. 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.

9.5. 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. 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

- Only for research use.
- 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. SUMMARY OF TEST PROCEDURE

SCHEME OF THE ASSAY
Chikungunya Virus IgM µ-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.

Assay Procedure

	Substrate Blank (A1)	Negative control	Cut-off control	Positive control	Sample (diluted 1+100)
Negative control	-	50µl	-	-	-
Cut-off control	-	-	50µl	-	-
Positive control	-	-	-	50µl	-
Sample (diluted 1+100)	-	-	-	-	50µl
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					
Reconstituted Antigen	-	50µl	50µl	50µl	50µl
Incubate for 30 min at room temperature (20-25 °C) Wash each well three times with 300µl of Washing Buffer					
Antibody Solution	-	50µl	50µl	50µl	50µl
Incubate for 30 min at room temperature (20-25 °C) Wash each well three times with 300µl of Washing Buffer					
Streptavidin conjugate	-	50µl	50µl	50µl	50µl
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					
TMB Substrate solution	100µl	100µl	100µl	100µl	100µl
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)					

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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instruc-tions d'utilisation	Consulte las Instruc-ciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precau-ciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore