SERION ELISA *classic* Chikungunya Virus IgG/IgM

SERION ELISA *classic* Chikungunya Virus IgG/IgM

Enzyme immunoassay for determination of human antibodies

For sale in the U.S. for Research Use Only. Not for diagnostic use.

SERION ELISA *classic* Chikungunya Virus IgG	Order no. ESR148G
SERION ELISA *classic* Chikungunya Virus IgM	Order no. ESR148M

1 INTENDED USE

The SERION ELISA *classic* Chikungunya Virus IgG and IgM tests are qualitative and quantitative immunoassay for the detection of human antibodies in serum or plasma directed against Chikungunya Virus.

For sale in the U.S. for Research Use Only. Not for diagnostic use.

2 BACKGROUND

Chikunguya Virus (CHIKV) is a member of the Alphavirus genus. It is also classified as an Arbovirus due to vector-dependent transmission by mosquitos. The enveloped virus carries structural glycoproteins E1-E3 which are immunogenic. Several non-structural proteins (nsP) and a ss-(+) RNA genome are present within the viral capsid.

Chikungunya fever is characterized by fever, joint pain and swelling, rash, and flu-like symptoms. Symptoms usually appear within a period of 1-2 weeks post-infection. The disease is characterized by a biphasic course with an initial stage of high virus load and a convalescent stage without viremia. In rare cases, neurological disorders like Guillain-Barré syndrome and meningoencephalitis have occurred.

3 TEST PRINCIPLE SERION ELISA *classic*

The ELISA (Enzyme-Linked Immunosorbent Assay) is an immunoassay suited to the detection of antibodies. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the SERION ELISA *classic* microtiter plate are coated with specific antigens of the pathogen of interest. If antibody in a sample is present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the antigen-antibody complex. The colorless substrate p-nitrophenylphosphate is then converted into the colored product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the antibody in the sample and is measured photometrically.

4 KIT COMPONENTS

Test Components	Pieces/ Volume
Break apart microtiter test strips each with eight antigen coated single wells, (altogether 96) MTP, 1 frame. The coating material is inactivated.	12 pieces
Standard serum (ready-to-use) STD, Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface Antigen) and anti-HCV Ab; Preservative: <0.1% sodium azide; coloring: Amaranth O	2 x 2 ml
Negative control serum (ready-to-use) NEG, Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface Antigen) and anti-HCV Ab; Preservative: <0.1% sodium azide; coloring: Lissamin Green V	2 ml
Anti-human IgA, IgG, or IgM conjugate (ready-to-use) APC Anti-human IgA, IgG or IgM polyclonal antibody, Conjugated to alkaline phosphatase, stabilized with protein stabilization solution; Preservative: <0.1% methylisothiazolone, <0.1% bromnitrodioxane	13ml
Washing solution concentrate (sufficient for 1000ml WASH, Sodium chloride solution with Tween 20 and 30mM Tris-HCl, pH 7.4; Preservative: <0.1% sodium azide	33.3ml
Dilution buffer (ready-to-use) DILB, Protein-containing phosphate buffer with Tween 20; Preservative: <0.1% sodium azide; coloring: 0.01g/l Bromphenol blue	2 x 50ml
Stopping solution (ready-to-use) STOP, <0.1N sodium hydroxide, 40mM EDTA	15ml
Substrate (ready-to-use) pNPP, Para-nitrophenylphosphate in solvent-free buffer; Preservative: <0.1% sodium azide	13ml
Quality control certificate with standard curve and evaluation table INFO, (quantification of antibodies in IU/ml or U/ml)	2 pages

5 MATERIAL REQUIRED BUT NOT SUPPLIED

- Common laboratory equipment
- For IgM detection, SERION Rf-Absorbent (Order no. Z200, 20ml)
- Photometer for microtiter plates with filter, wavelength 405nm, recommended reference wavelength 620nm-690nm (e.g., 650nm)
- Microtiter plate washer
- Incubator 37°C
- Moist chamber
- Distilled water
- Optional: SERION ELISA *control*

6 STORAGE AND STABILITY

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	Unopened After opening at 2-8°C in closed aluminum bag with desiccant	See expiry date 6 months
Control sera / Standard sera	Unopened After opening at 2-8°C	See expiry date 6 months
Conjugate	Unopened After opening at 2-8°C	See expiry date 6 months
Dilution buffer	Unopened after opening at 2-8°C	See expiry date 6 months
Washing solution	Unopened / after opening at 2-8°C Working dilution at 2-8°C Working dilution at room temperature	See expiry date 2 weeks 1 week
Substrate	Unopened After opening at 2-8°C	See expiry date 6 months
Stopping solution	Unopened After opening at 2-8°C	See expiry date 6 months

7 TEST PROCEDURE SERION ELISA *classic*

7.1 Evidence of Deterioration

Optimum results can only be achieved if the instructions are strictly followed. Only use SERION ELISA *classic* reagents when using SERION ELISA *classic* immunoassays. The components must not be exchanged for reagents of other manufacturers. Standard and control sera of SERION ELISA *classic* immunoassays are defined exclusively for the test kit to be used and must not be used in other lots. Washing solution, substrate and stop solution can be used for all SERION ELISA *classic* immunoassays irrespective of the lot and the test.

Each SERION ELISA *classic* test contains a ready-to-use sample dilution buffer. In some cases the use of special dilution buffers is necessary to guarantee consistent quality and reliable results. The dilution buffers can be used irrespective of the lots.

There are three different conjugate concentrations for each immunoglobulin class (IgA, IgG, IgM) indicated on the label as + (low), ++ (medium), and +++ (high). Conjugates with the same concentration and of the same immunoglobulin class are interchangeable and can be used for other SERION ELISA *classic* immunoassays irrespective of the lot and the test. Dilution or alteration of the reagents may result in a loss of sensitivity. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination.

Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the vials containing control sera before use and also all samples after dilution (e.g., by using a vortex mixer).

Be sure to pipette carefully and comply with the given incubation times and temperatures. Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate or substrate can result in different pre-incubation times, which may influence the precision and reproducibility of the results. Avoid exposure of reagents to strong light during storage and incubation.

Adequate washing avoids test unspecificities. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!

Reagents must be tightly closed after use to avoid evaporation and contamination. Take care not to mix up the caps of the bottles and/or vials.

The SERION ELISA *classic* immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

7.2 Sample Preparation and Storage

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

7.2.1 Dilution of Samples

Before running the test, samples (V_1) must be diluted in dilution buffer (V_2) as follows:

SERION ELISA *classic* Chikungunya Virus IgG

$V_1 + V_2$ = 1:500	add	10ul	sample
	each to	1000ul	dilution buffer (= 1:100)
		50ul	of the first dilution
	each to	200ul	dilution buffer (= 1:5)

After dilution and before pipetting into the microtiter plate, the samples must be mixed thoroughly to prepare a homogenous solution.

Interference by rheumatoid factors

Rheumatoid factors are autoantibodies mainly of the IgM class which may bind to IgG immune complexes. The presence of non-specific IgM rheumatoid factors can lead to false-positive results in the IgM assay. In addition, it is possible that weak-binding pathogen-specific IgM antibodies may be displaced by stronger-binding IgG antibodies which can cause a false-negative IgM result. Therefore, it is necessary to pre-treat samples with rheumatoid factor absorbent prior to IgM detection (SERION Rf-Absorbent, order no. Z200, 20ml/100 tests). Rf-absorption is performed by incubating sample in Rf-Absorbent for 15 minutes at room temperature or overnight at 4°C.

Before running the ELISA test, rheumatoid factor-absorbent (V_1) must be diluted 1:5 in dilution buffer (V_2).

V1 + V2 = V3 (1:5)	add	200ul	Rf-absorbent
	each to	800ul	dilution buffer

Samples (V_4) must be diluted in this Rf-dilution buffer (V_3):

V4 + V3 = 1:100	add	10ul	sample
	each to	1000ul	Rf-dilution buffer

After dilution and before pipetting into the microtiter plate, samples must be mixed thoroughly to prepare a homogenous solution.

7.2.2 Sample Storage

Samples should not be stored for more than 7 days at 2-8°C. Extended storage is possible at ≤ -20°C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2-8°C for one week.

7.3 Preparation of Kit Reagents

Bring all reagents to room temperature before testing.

7.3.1 Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate, please cut off the top of the marked side only in order to guarantee proper resealing. Take unrequired wells out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

7.3.2 Negative Control Sera / Standard Sera (ready-to-use)

Negative control and standard sera are ready-to-use and must not be diluted any further. For each test run (independent of the number of microtiter test strips to be used) negative control and standard sera must be included. Standard sera should be set up in duplicate. Do not treat negative control and standard sera with Rf-absorbent.

7.3.3 Anti-human IgA, IgG, or IgM AP-Conjugate (ready-to-use)

The required conjugate concentration (i.e., +, ++, +++) is indicated on the quality control certificate. Please refer also to the specification on the label. Avoid contamination.

7.3.4 Washing Solution (Concentrate)

Dilute washing buffer concentrate (V_1) 1:30 with distilled H_2O to a final volume of V_2. Bottles used for the working dilution should be cleaned regularly. Discard cloudy solutions.

Example:

Buffer concentrate (V_1)	**Final volume (V_2)**
33.3ml	1000ml
1.0ml	30ml

7.3.5 Dilution Buffer for Samples (ready-to-use)

Discard cloudy solutions.

7.3.6 Substrate (ready-to-use)

Substrate in unopened bottle may have a slight yellow color which does not reduce the quality of the product! Avoid contamination.

7.3.7 Stopping Solution (ready-to-use)

7.4 Overview – Test Procedure

SERION ELISA *classic*
Chikungunya Virus IgG/IgM quantitative

For IgM detection, absorption of rheumatoid factor, see Section 7.2.1;
incubation for 15 minutes at room temperature or overnight at 4°C.

sample dilution[1]
IgG 1:500 IgM 1:100

Pipette diluted samples and ready-to-use negative control/
standard sera into the microtiter wells (100ul/well)

⇩

INCUBATION 60 min / 37°C
moist chamber

⇩

WASH (4 x 300ul/well DIL WASH)[2]

⇩

Pipette conjugate solution APC (100ul/well)

INCUBATION 30 min / 37°C
moist chamber

⇩

WASH (4 x 300ul/well DIL WASH)[2]

⇩

Pipette substrate solution pNPP (100ul/well)

⇩

INCUBATION 30 min / 37°C
moist chamber

⇩

Pipette stopping solution STOP (100ul/well)

⇩

READ EXTINCTION at 405nm

[1]Special dilution buffers for the following SERION ELISA classic tests:
Borrelia burgdorferi IgG, IgM and EBV EA IgG.

[2]For manual use: tap plate at the end of the wash procedure on paper towel.

7.5 Manual Test Procedure

1. Place the required number of **wells in the frame** and prepare a protocol sheet.
2. Add each **100ul of diluted sample or ready-to-use negative control / standard sera** into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

Well	Quantitative ELISA
A1	Substrate blank
B1	Negative control
C1	Standard serum
D1	Standard serum
E1	Sample 1 . . .
F1	Sample 2 . . .

3. **Sample incubation** for 60 minutes (+/- 5 min) at 37°C (+/- 1°C) in moist chamber.
4. After incubation **wash** all wells with washing solution (by automated washer or manually):
   - aspirate or shake out the incubation solution
   - fill each well with 300ul washing solution
   - aspirate or shake out the washing solution
   - repeat the washing procedure 3 times (altogether 4 times!)
   - dry by tapping the microtiter plate on a paper towel
5. **Addition of conjugate**
   Add 100ul of the ready-to-use IgG/IgM conjugate to the appropriate wells (except substrate blank).
6. **Conjugate incubation** for 30 minutes (+/- 1 min) at 37°C (+/- 1°C) in moist chamber.
7. After incubation **wash** all wells with washing solution (see above).
8. **Addition of substrate**
   Add 100ul of ready-to-use substrate solution to each well (including well for substrate blank!)
9. **Substrate incubation** for 30 minutes (+/- 1 min) at 37°C (+/- 1°C) in moist chamber. Ensure incubation is in the dark.
10. **Stopping the reaction**
    Add 100ul of stopping solution to each well, shake microtiter plate gently to mix.
11. **Read extinction**
    Read optical density (OD) within 60 minutes at 405nm against substrate blank, reference wavelength between 620nm and 690nm (e.g. 650nm).

7.6 Automated Test Procedure

SERION ELISA are suited for use with Immunomat using the following consumables: VT124, VT111, VT112. They are also suited for processing on similar analyzers. For processing on the Immunomat, the current software version including reagent check has to be used. The automated processing is performed analogous to manual use. Please note that under special working conditions (e.g. ambient temperature), internal laboratory adaptations of the substrate incubation times may be necessary.

8 TEST EVALUATION

8.1 SERION ELISA *classic* Chikungunya Virus IgG/IgM

The mathematical curve fitting for antibody quantification with SERION ELISA *classic* immunoassays is based on the 4-parameter logistic (4 PL) function.

$$Activity\ (U/ml) = e^{C-\frac{1}{B}\ln\left(\frac{D-A}{OD(Patient)*F-A}-1\right)}$$

The 4 parameters A, B, C, and D are representative for the exact shape of the standard curve:

Parameter A:	Lower asymptote (OD)
Parameter B:	Slope of the curve
Parameter C:	Inflection point
Parameter D:	Upper asymptote (OD)

Institut Virion\Serion GmbH establishes a lot-specific 4 PL standard curve for each SERION ELISA *classic* immunoassay in multiple test runs under optimal test conditions. The four parameters are indicated on the quality control certificate of each individual SERION ELISA *classic* test.

For the adaptation of the test level to the given 4 PL standard curve, the correction factor F is calculated by dividing the standard reference OD value indicated on the quality control certificate with the measured, and consequently test run-specific, standard OD value.

$$F = \frac{STD\ reference\ OD\ value}{measured\ \ STD\ OD\ value}$$

By multiplying the OD values obtained from samples with the correction factor F, the level of each individual test run is adjusted to the given 4 PL standard curve. Thereby, interassay deviations are compensated for and antibody activities can be directly evaluated from the 4 PL standard curve.

After subtraction of the substrate blank from all measured OD values and calculation of the mean OD value of the standard serum (STD), tested in duplicate, the evaluation of antibody activities from the optical measurement signals (OD) of samples can be performed with 4PL function presented above.

8.2 Automated Evaluation / Software

For automated evaluation of optical measurement signals (OD), the software SERION easy*ANALYZE* as well as Microsoft® Excel®-based software tool SERION*activity* are available on request.

8.3 Borderline Ranges

The borderline ranges of the SERION ELISA *classic* Chikungunya Virus IgG/IgM test are specified on the quality control certificates and indicate the range of borderline test results. Values below this range indicate a negative result; values above the borderline range indicate a positive result.

8.4 Limits of Quantification

The limits of quantification are specified on the quality control certificate of the SERION ELISA *classic* Chikungunya Virus IgG/IgM. The linearity of dilution within this range has been demonstrated in comprehensive evaluation studies. If a sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.

8.5 Qualitative Evaluation with SERION ELISA *classic* Chikungunya Virus IgG/IgM

For the SERION ELISA *classic* test evaluation, a lot-specific quality control certificate with standard curve and an evaluation table is included in the test kit so that the obtained OD values may be assigned to the corresponding antibody activities. The substrate blank must be subtracted from all OD values prior to evaluation. Mean OD value of the standard serum (STD), tested in duplicate, has to be used.

Method 1:

In the first line of the evaluation table, several ranges of OD values for the standard serum that cover the entire standard validity range are provided. Choose the column that corresponds to the measured mean OD value obtained for the standard serum. This column contains the upper and lower cut-off OD values for evaluation of antibody in the sample. OD values below the lower cut-off are considered negative values, and OD values above the upper cut-off are considered positive values. Use of the correction factor F is not needed for this evaluation method.

Method 2:

To fix the cut-off ranges, multiply the mean value of the OD measured for the standard serum with the numerical data of the quality control certificate (see special case formulas), e.g.:

OD = 0.502 x MW(STD) with upper cut-off
OD = 0.352 x MW(STD) with lower cut-off
Calculation example:
Standard serum mean OD = 0.64
Upper cut-off: OD = 0.502 x 0.64 = 0.321
Lower cut-off: OD = 0.352 x 0.64 = 0.225

If the measured OD of the standard serum is 0.64, the range of the cut-off is between 0.225-0.321.

8.6 Criteria of Validity

- The substrate blank must be <0.25 OD.
- The negative control must be negative.
- The mean OD value (after subtraction of the substrate blank!) of the standard serum must be within the validity range which is given on the lot-specific quality control certificate.
- The variation of OD values of the standard serum or cut-off serum must not be higher than 20%.

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

9 PERFORMANCE CHARACTERISTICS

9.1 Cross-reactivities

SERION ELISA *classic* Chikungunya Virus IgG
Potential cross-reactivities of the SERION ELISA *classic* Chikungunya Virus IgG were evaluated with known positive sera (10 each) for Zika Virus IgG, Dengue Virus IgG, Rubella Virus IgG, TBE Virus IgG, Influenza A Virus IgG, Epstein-Barr Virus VCA IgG, West Nile Virus IgG, Parvovirus B19 IgG, Measles Virus IgG, Mumps Virus IgG, Varicella-Zoster Virus IgG, and Influenza B Virus IgG as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Cross-reactivity with ten Zika Virus IgG sera were observed. Other cross-reactivities cannot be ruled out in general.

SERION ELISA classic Chikungunya Virus IgM
Potential cross-reactivities of the SERION ELISA *classic* Chikungunya Virus IgM were evaluated with known positive sera (10 each) for Zika Virus IgM, Dengue Virus IgM, Rubella Virus IgM, Cytomegalovirus IgM, Epstein-Barr Virus VCA IgM, West Nile Virus IgM, Parvovirus B19 IgM, Measles Virus IgM, Mumps Virus IgM, and Varicella-Zoster Virus IgM as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Cross-reactivity with four Zika Virus IgM, one TBE Virus IgM, one Epstein-Barr Virus VCA IgM, and three Parvovirus B19 IgM were observed. Other cross-reactivities cannot be ruled out in general.

9.2 Interfering substances

SERION ELISA classic Chikungunya Virus IgG/IgM
No interferences have been detected for sera with concentrations up to 2.00g/L hemoglobin, 11.50g/L lipemia/triglyceride, or 0.201g/L bilirubin (conjugated and unconjugated).

10 SAFETY MEASURES

10.1 Statements of Warning

The SERION ELISA *classic* is designed for use by qualified personnel who are familiar with good laboratory practice. All kit reagents and human samples should be handled carefully using established good laboratory practice.

- This kit contains human blood components. Although all control- and cut-off sera have been tested and found negative for anti-HIV Ab, HBs-Ag (*Hepatitis B Virus surface Antigen*) and anti-HCV Ab, they should be considered potentially infectious.
- Do not pipette by mouth.
- Do not smoke, eat, or drink in areas in which samples or kit reagents are handled.
- Wear disposable gloves, laboratory coat, and safety glasses while handling kit reagents or samples. Wash hands thoroughly afterwards.
- Samples and other potentially infectious material should be decontaminated after use.
- Reagents should be stored safely and be inaccessible to unauthorized access, e.g. children.

10.2 Disposal
Please observe the relevant statutory requirements!

11. LITERATURE REFERENCES

1. Cherabuddi K et al. 2016 Zika and Chikungunya virus co-infection in a traveler returning from Colombia, 206: virus isolation and genetic analysis. JMM Case Rep. 206 Dec 19; 3(6): 3005072.

2. Sardi SI et al. 2016. Coinfections of Zika and Chikungunya Viruses in Bahia, Brazil, Identified by Metagenomic Next-Generation Sequencing. J Clin Microbiol 2016 Sep; 54(9): 2348-53.

3. Villamil-Gomez WE et al. 2016 Zika, dengue, and chikungunya co-infection in a pregnant woman from Colombia. Int J Infect Dis 2016 Oct; 51L 135-138.

4. Göetz GP et al. 2017 Mosquito co-infection with Zika and chikungunya virus allows simultaneous transmission without affecting vector competence of Aedes aegypti. PLoS Negl Trop Dis 2017 Jun 1; 11(6): e0005654.

5. Waggoner JJ et al. 2016 Viremia and Clinical Presentation in Nicaraguan Patients Infected With Zika Virus, Chikungunya Virus, and Dengue Virus. Clin Infect Dis 2016 Dec 15; 63(12): 1584-1590.

6. Pessôa R et al. 2016 Investigation Into an Outbreak of Dengue-like Illness in Pernambuco, Brazil Revealed a Cocirculation of Zika, Chikungunya, and Dengue Virus Type 1. Medicine (Baltimore) 2016 Mar; 95(12): e3201.