RIDASCREEN® Chikungunya Virus IgG capture,
RIDASCREEN® Chikungunya Virus IgM µ-capture

REF K8121
K8131

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1. Intended use

For *in vitro* diagnostic use. The RIDASCREEN® Chikungunya Virus tests are enzyme immunoassays for the qualitative detection of IgG or IgM antibodies to the Chikungunya virus in human serum or plasma. The tests are intended for use in cases of suspected Chikungunya virus infection.

2. Summary and explanation of the test

Chikungunya viruses are enveloped, single-strand RNA viruses of the genus Alphavirus in the Togaviridae family. They have a diameter of approximately 60 nm. There are five different variants of the virus, based on geographic distribution: West African, Central African, East/South African, Indian Ocean and Asian.
Chikungunya viruses are transmitted to humans mainly by mosquitoes of the Aedes aegypti or Aedes albopictus species.
In humans, the virus causes Chikungunya fever. After a 1 - 6 day incubation period, the illness begins with the abrupt onset of joint pain, frequently with biphasic fever, myalgia and nausea. The fever generally lasts just a few days. Other symptoms frequently develop as the illness progresses, including swollen lymph nodes, maculopapular exanthema, headache and exhaustion. The illness normally abates after approximately two weeks, but joint pain can persist for months. Hemorrhagic complications can develop in very rare cases. Lasting damage and death are rare. Alphavirus infections are uncommon in Europe, but imported or travel cases can arise.
As a result of the immune system’s response, the body produces specific antibodies to the pathogen after being infected with the Chikungunya virus. These antibodies can be detected in the serum or plasma with the help of immunological methods. The selection of the pathogen-specific antigen used and the applied test method are important for the validity of a test.

3. Test principle

Anti-human IgG or IgM antibodies are bound on a microtiter plate. IgG or IgM antibodies present in patient specimens bind to the anti-human IgG or IgM antibodies. Unbound specimen material is removed by washing. The Chikungunya virus antigens are then deposited on the plate. A wash step follows and then biotinylated Chikungunya virus antibodies are added. After another wash step, enzyme-marked Streptavidin is pipetted, which binds to the immobilized Chikungunya-specific immune complexes. The enzyme changes a colorless substrate (H_2O_2/TMB) into a blue end product. The enzymatic reaction is stopped by the addition of sulfuric acid. This addition causes the color to change from blue to yellow. The final measurement is taken in a photometer at 450 nm (reference wavelength ≥ 620 nm).

4. Reagents provided

Tab. 1: Reagents provided (The reagents in a kit are sufficient for 96 assays.)

			K8121 IgG	K8131 IgM
Plate	96 assays	Microwell plate, 12 microwell strips (which can be divided) in the frame; coated with anti-human IgG antibodies	X	
Plate	96 assays	Microwell plate, 12 microwell strips (which can be divided) in the frame; coated with anti-human IgM antibodies		X
Diluent	100 ml	Sample diluent, ready-to-use; phosphate buffer, colored yellow	X	X
Wash	50 ml	Wash buffer, 20X concentrated; phosphate buffer	X	X
Control IgG | + *Red lid*	1.5 ml	Positive control IgG, ready-to-use; diluted human serum or plasma; colored yellow	X	
Control IgG | – *Blue lid*	1.5 ml	Negative control IgG, ready-to-use; diluted human serum or plasma; colored yellow	X	
Cut-off IgG *Green lid*	2 ml	Cut-off control IgG, ready-to-use; diluted human serum or plasma; colored yellow	X	
Control IgM | + *Red lid*	1.5 ml	Positive control IgM, ready-to-use; diluted human serum or plasma; colored yellow		X
Control IgM | – *Blue lid*	1.5 ml	Negative control IgM, ready-to-use; diluted human serum or plasma; colored yellow		X
Cut-off IgM *Green lid*	2 ml	Cut-off control IgM, ready-to-use; diluted human serum or plasma; colored yellow		X
Conjugate IgG *Black lid*	6 ml	Streptavidin conjugate, ready-to-use; Peroxidase conjugate Streptavidin, colored blue	X	
Conjugate IgM *Black lid*	6 ml	Streptavidin conjugate, ready-to-use; Peroxidase conjugate Streptavidin, colored red		X
Ag LYO *Red lid*	1 ml	Chikungunya antigen, lyophilized; can be used in 1 ml ready-to-use wash buffer after reconstitution	X	X
Ab SOLN *White lid*	6 ml	Chikungunya antibody solution, ready-to-use; biotinylated Chikungunya virus antibody solution, colored blue	X	X
Substrate *Yellow lid*	15 ml	Substrate, ready-to-use; H_2O_2/tetramethylbenzidine	X	X
Stop *Red lid*	15 ml	Stop solution, ready-to-use; 0.2 mol/l sulfuric acid	X	X

Information on hazardous substances complies with the labeling requirement. For further details, see the safety data sheets (SDSs) at www.r-biopharm.com .

5. Storage instructions

The test kit can be used up to the expiration date printed on the label when stored at 2 - 8 °C. The diluted wash buffer has a shelf-life of four weeks when stored at 2 - 8 °C and five days when stored at room temperature (20 - 25 °C). After the expiration date, the quality guarantee is no longer valid.
Open the aluminum bag containing the microtiter plate without separating the clip seal. Store unneeded microtiter strips in the closed aluminum bag.
Prevent contamination of the reagents, and prevent direct light from shining on the colorless substrate.

6. Reagents required but not provided

6.1. Reagents

– Distilled or deionized water

6.2. Accessories

– Specimen vials
– Incubator 37 °C
– Vortex mixer
– Micropipettes for volumes of 10 - 100 µl and 100 - 1000 µl
– Graduated cylinder (1000 ml)
– Washer for microtiter plates or multichannel pipettes
– Photometer for microtiter plates (450 nm, reference filter ≥ 620 nm)
– Filter paper (lab wipes)
– Waste container with a 0.5% hypochlorite solution

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

Only trained laboratory personnel may perform this test. Follow the guidelines for working in medical laboratories. The instructions for use for performing this test must be strictly followed. Do not pipette specimens or reagents using your mouth. Avoid contact with broken skin or mucous membranes. Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and specimens, and wash hands after completing the test. Do not smoke, eat, or drink in areas where specimens are handled.

For further details, see the safety data sheets (SDSs) at www.r-biopharm.com.

The control sera found in the kit (positive control, cut-off control, and negative control) were tested for HIV Ab, HCV Ab, and HBsAg and were negative. Still, they should be treated as potentially infectious, as should the patient specimens and all

materials that you touch, and they should be handled according to the respective national safety regulations.

Users are responsible for proper disposal of all reagents and materials after use. Follow the respective national disposal regulations.

8. Collection and storage of specimens

This test was developed for the examination of human serum and plasma specimens. Following blood collection, the serum should be separated from coagulum as quickly as possible to prevent hemolysis. The specimens should be kept cool or frozen until testing. Repeated freezing and thawing of specimens must be avoided, as must microbial contamination. The use of heat-inactivated, lipemic, hemolytic, icteric, or cloudy specimens can lead to false results.

Tab. 2: Specimen storage

Undiluted serum or plasma		Diluted serum or plasma
2 - 8 °C	-20 °C	2 - 8 °C
1 week	> 1 week	7 hours

9. Test procedure

9.1. General information

Prior to use, bring all reagents and microtiter strips to room temperature (20 - 25 °C). Once they have reached room temperature, remove the microtiter strips from the aluminum bag. Mix the reagents well immediately before use. After use, store the kit promptly at 2 - 8 °C again.
Take only as many reagents as needed for performing the test. Do not return extra reagent to the container as this can lead to contamination.
Microtiter strips may not be used more than once. Do not use reagents or microtiter strips if the packaging is damaged or the containers are not tightly sealed.

9.2. Preparing the wash buffer

Mix 1 part wash buffer concentrate wash Wash with 19 parts distilled water. To do so, add 50 ml concentrate to a 1000-ml cylinder and fill with distilled water to 1000 ml. Any crystals present in the concentrate should be dissolved beforehand through heating (water bath at 37 °C). The diluted wash buffer will last for five days when stored at room temperature (20 - 25 °C).

9.3. Preparation of Chikungunya antigen solution

Dissolve the contents of each vial in 1 ml of diluted wash solution by shaking. The solution must be incubated for 15 min at room temperature. The ready-to-use solution will last for one day when stored at 2 - 8 °C.

9.4. Specimen preparation

Prior to the start of the test, dilute the serum or plasma specimens 1 + 100 with the sample diluent Diluent .

For example, 10 µl serum/plasma + 1 ml diluent Diluent

Attention!
The cut-off control, negative control, and positive control are ready-to-use and should not be diluted.

9.5. First incubation

After a sufficient number of wells have been placed in the frame, pipette 50 µl each of the diluted sera, ready-to-use controls into the respective wells; position A1 (substrate blank value) remains empty.
Use the appropriate controls (IgG, IgM). It is recommended to perform the cut-off control in duplicate. The plate will be incubated in an incubator for 60 minutes at 37 °C. The base of the wells should not be in contact with materials that easily conduct heat. The microtiter plate must be covered with the provided protective foil during incubation.

A1	Substrate blank value
B1	Negative control
C1	Cut-off control
D1	Cut-off control
E1	Positive control
F1, G1	Patient serum 1, 2, etc.

Attention!
Do not place the microtiter plate in a cold incubation container that will not be heated to 37 ° until during the incubation. The container must be adapted to 37 °C beforehand.

9.6. Washing

Empty the wells into a waste container that has hypochlorite solution for disinfection. Next tap the plate over absorbable paper to remove the remaining moisture. Next wash the plate three times using 300 µl wash buffer each time. After every wash, tap the plate over an unused area of paper to ensure complete emptying.

When a washing machine is used, make sure that the machine is correctly set to the plate type. After washing, tap the plate over absorbable, clean paper to remove residual moisture.

9.7. Second incubation

Add 50 µl of the prepared Chikungunya antigen solution Ag LYO [Ag LYO] to all wells, except A1 (substrate blank value). Next incubate the plate for 30 minutes at room temperature (20 - 25 °C). The microtiter plate must be covered with the provided protective foil during incubation.

9.8. Washing

Wash three times as described in item 9.6.

9.9. Third incubation

Add 50 µl of the Chikungunya antigen solution Ab SOLN [Ab SOLN] to all wells, except A1 (substrate blank value). Next incubate the plate for 30 minutes at room temperature (20 - 25 °C). The microtiter plate must be covered with the provided protective foil during incubation.

9.10. Washing

Wash three times as described in item 9.6.

9.11. Fourth incubation

Add 50 µl Streptavidin conjugate [Conjugate] to all wells except A1 (substrate blank value). Next incubate the plate for 30 minutes at room temperature (20 - 25 °C). The microtiter plate must be covered with the provided protective foil during incubation.

9.12. Washing

Wash three times as described in item 9.6.

9.13 Fifth incubation

Add 100 µl substrate [Substrate] to all wells, including A1 (substrate blank value). Next incubate the plate for 15 minutes in the dark at room temperature (20-25 °C). Then add 100 µl stop reagent stop [Stop] to all wells to stop the reaction, which changes the color from blue to yellow. After carefully mixing the plate (by gently tapping on edge of plate), measure the extinction in a plate photometer at 450 nm (reference wave length ≥ 620 nm) within 30 minutes. The null value comparison is made against the substrate blank value (position A1).

10. Quality control - indication of instability or deterioration of reagents

For quality control, the positive, negative, and cut-off controls must be performed every time the test is carried out. The cut-off control is performed in duplicate, and the average value determined from the two individual measurements. The test has been carried out correctly when the extinction values (OD) of the controls meet the following criteria:

Tab. 3: Criteria for quality control

	OD
Substrate blank value	< 0.100
Negative control	< cut-off
Cut-off control	0.150 - 1.300
Positive control	> cut-off

A deviation from the expected values as well as cloudiness or blue coloring of the colorless substrate prior to addition to the wells can be an indication of an expired reagent.

If the specified values are not met, check the following before repeating the test:
- Expiration date of the reagents used
- Functional performance of the equipment used (e.g., calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks; a blue-colored substrate solution should no longer be used.

If the conditions are still not met after the test is repeated, please contact the manufacturer.

11. Evaluation and interpretation

11.1. Calculation of the specimen index

1 The average extinction value of the cut-off control will be calculated.
2 Divide the extinction of the patient specimen by the calculated average extinction value of the cut-off control.

For example:
Cut-off control 1 OD = 0.440
Cut-off control 2 OD = 0.420
Average value: = 0.430
Specimens OD = 1.591

$$\text{Specimen index} = \frac{1.591}{0.430} \text{x} 10 = 37$$

Tab. 4: Analysis of specimen index

	Negative	Borderline	Positive
Specimen index	< 9	09 - 11	> 11

12. Limitations of the method

The RIDASCREEN® Chikungunya Virus ELISA detects IgG and IgM antibodies against the Chikungunya virus. A connection between the amount of the measured extinction value and the presence or severity of clinical symptoms cannot be derived. The obtained results must always be interpreted in connection with the clinical signs and symptoms.

A negative result does not rule out an existing infection. At an early stage of infection, antibody production can still be so low that the antibody test will be negative. If clinical suspicion is present, a follow-up serum sample should, therefore, be tested.

Generally in serological tests, two consecutive sera of the patient should be tested to improve diagnostic validity. Important for the interpretation of a finding is the course of the titer.

A positive result does not rule out the presence of another infectious pathogen as the cause of a disease.

Cross-reactivities with antibodies against Borrelia, CMV, Toxoplasma, EBV and other alphaviruses cannot be excluded.

13. Performance characteristics

Tab. 5: Inter-assay variance (n = 12)

Inter-assay variance	IgG		IgM	
	U/ml	CV	U/ml	CV
Serum 1	42.7	7.2 %	32.4	4.9 %
Serum 2	58.3	8.5 %	27.0	5.8 %
Serum 3	2.9	9.6 %	6.0	12.8 %

Tab. 6: Intra-assay variance (n = 24)

Intra-assay variance	IgG		IgM	
	OD	CV	OD	CV
Serum 1	0.384	7.9 %	0.287	8.5 %
Serum 2	1.405	5.2 %	0.769	5.8 %
Serum 3	1.877	6.3 %	0.618	5.5 %

Tab. 7: Sensitivity and specificity

	IgG	IgM
Sensitivity	98.7 %	100 %
Specificity	100 %	100 %

14. Version history

Version number	Chapter and designation
2017-12-13	Release version

15. Explanation of symbols

General symbols

IVD	For in vitro diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Testspecific symbols

Plate	Microwell plate
Diluent	Sample diluent
Wash	Wash buffer, 20x
Control IgG | +	Positive control IgG
Control IgG | -	Negative control IgG
Cut-off IgG	Cut-off control IgG
Control IgM | +	Positive control IgM
Control IgM | –	Negative control IgM
Cut-off IgM	Cut-off control IgM
Conjugate IgG	Streptavidin conjugate IgG
Conjugate IgM	Streptavidin conjugate IgM
Ag LYO	Chikungunya antigen, lyophilized
Ab SOLN	Chikungunya antibody solution
Substrate	Substrate
Stop	Stop solution

16. References

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