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

REF K8421
K8431

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1. Intended use

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

The tests are intended to be used in cases of suspected Zika virus infection.

2. Summary and explanation of the test

The Zika virus is a single-stranded RNA virus of the Flaviviridae family, genus Flavivirus.
Zika viruses are primarily transmitted by mosquitoes belonging to the species *A. aegypti* and *A. albopictus*. There are, however, reports of less common types of transmission, including blood transfusions, perinatal transmission, and sexual contact.
The precise incubation period of Zika fever is not known, but is probably around a few days.
It is estimated that only one of five people infected with the Zika virus will develop symptoms. Clinical manifestations are similar to those of dengue virus or chikungunya virus infection, but normally have a milder course.
The most common symptoms are maculopapular rash, mild fever, arthralgia, myalgia, headache, and conjunctivitis. Less common symptoms are edemas, neck pain, cough, vomiting, and hematospermia. Infections in humans are usually mild and self-limiting, and the symptoms disappear spontaneously usually within three to seven days. Arthralgia can last up to one month. In rare cases, Guillain-Barré syndrome (GBS), a disease of the peripheral nervous system, can also develop following infection with the Zika virus.
A correlation between Zika virus infection in pregnancy and brain defects in the unborn child is now considered to be likely.
As a result of the immune system's response, the body produces specific antibodies to the pathogen after being infected with the Zika virus. These antibodies can be detected in the serum 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 and anti-human 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. Next the enzyme-marked Zika virus antigens are added. This antigen conjugate binds to the specific bound IgG or IgM antibodies. In a second wash step, unbound conjugate Conjugate is removed. 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.)

			K8421 IgG	K8431 IgM
Plate	96 assays	Microtiter plate; 12 microtiter strips (separable) in strip holder; coated with anti-human IgG antibodies	X	
Plate	96 assays	Microtiter plate; 12 microtiter strips (separable) in strip holder; 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*	2 ml	Positive control IgG, ready-to-use; diluted human serum or plasma; colored yellow	X	
Control IgG | – *Blue lid*	2 ml	Negative control IgG, ready-to-use; diluted human serum or plasma; colored yellow	X	
Cut-off IgG *Green lid*	3 ml	Cut-off control IgG, ready-to-use; diluted human serum or plasma; colored yellow	X	
Control IgM | + *Red lid*	2 ml	Positive control IgM, ready-to-use; diluted human serum or plasma; colored yellow		X
Control IgM | – *Blue lid*	2 ml	Negative control IgM, ready-to-use; diluted human serum or plasma; colored yellow		X
Cut-off IgM *Green lid*	3 ml	Cut-off control IgM, ready-to-use; diluted human serum or plasma; colored yellow		X
Conjugate IgG *Black lid*	15 ml	Zika virus conjugate; ready-to-use; peroxidase conjugate Zika virus antigen, colored blue	X	
Conjugate IgM *Black lid*	15 ml	Zika virus conjugate; ready-to-use; peroxidase conjugate Zika virus antigen, colored red		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. Manufacturing 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. Specimen preparation

The serum or plasma specimens being tested will be diluted with the sample diluent Diluent 1 + 100 prior to the start of the test.

For example, 10 µl serum + 1 ml diluent Diluent

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

9.4. First incubation

After a sufficient number of wells have been placed in the strip holder, pipette 100 µl each of the diluted sera, ready-to-use controls Control – , Control + , and cut-off Cut-off into the respective wells; position A1 (substrate blank value) remains empty. It is recommended to perform the cut-off control Cut-off 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 film 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.

9.5. 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.

9.6. Second incubation

Add 100 µl conjugate Conjugate to the respective wells except for A1 (substrate blank value). Next incubate the plate in an incubator for 30 minutes at 37 °C (see item 9.4.).

9.7. Washing

Wash three times as described in item 9.5.

9.8. Third incubation

Add 100 µl substrate Substrate to all wells, including A1 (substrate blank value). Next incubate the plate for exactly 15 minutes in the dark at room temperature (20 - 25 °C). Then add 100 µl stop solution Stop to all wells to stop the reaction, which will cause the color to change from blue to yellow. After carefully mixing the plate (by gently tapping on edge of plate), the extinction will be measured in a plate photometer at 450 nm (reference wave length ≥ 620 nm). 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 mean 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
Specimen	OD = 1.591

$$\text{Proben-Index} = \frac{1{,}591}{0{,}430} \text{x} 10 = 37$$

Tab. 4: Analysis of specimen index

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

12. Limitations of the method

RIDASCREEN® Zika Virus IgG capture and IgM µ-capture ELISA detect IgG or IgM antibodies to Zika viruses. 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.

Testing a specimen panel using antibody activities against potentially cross-reacting parameters cannot detect significant indications of false-positive results due to cross reactivities. However, in endemic areas, double infections and past infections with other flaviviruses should be taken into consideration.

13. Performance characteristics

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

Inter-assay variance	IgG		IgM	
	U/ml	CV	U/ml	CV
Serum 1	32.6	6.2%	25.2	10.7%
Serum 2	22.6	5.7%	11.4	5.3%
Serum 3	23.8	5.9%	6.8	8.8%

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

Intra-assay variance	IgG		IgM	
	OD	CV	OD	CV
Serum 1	0.422	6.7%	1.012	4.0%
Serum 2	1.062	2.1%	0.488	3.0%
Serum 3	0.988	2.6%	0.431	1.8%

Tab. 7: Sensitivity and specificity

	IgG	IgM
Sensitivity	96.0%	100%
Specificity	99.6%	98.6%

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	Microtiter 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	Zika virus conjugate IgG
Conjugate IgM	Zika virus conjugate IgM
Substrate	Substrate
Stop	Stop solution

16. References

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