Human Zika Virus IgM μ-capture ELISA Kit

Catalog #: NB-E400926M (96 wells)

User Manual

This kit is designed to quantitatively detect the levels of Human Dengue Virus IgG in serum/ plasma and other suitable sample solution.

Manufactured and Distributed by:

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Human Dengue fever IgG ELISA kit	NG-E10001
Human Dengue fever IgM ELISA kit	NG-E10002
Human Dengue Virus IgM μ-capture ELISA Kit	NN-E10001
Human Dengue Fever IgG/IgM ELISA Kit	NR-R10181
Dengue virus NS1 antigen ELISA kit	NR-R10004
Zika Virus NS1 Antigen ELISA Kit	NB-E400926
Zika Virus Envelope (Env) Antigen ELISA Kit	NB-E400927
Human Zika Virus IgG ELISA Kit	NB-400926G
Human Zika Virus IgM μ-capture ELISA Kit	NB-400926M

FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

1. INTRODUCTION

Zika Virus (ZIKV) is a single-stranded RNA virus of the *Flaviviridae* family (genus *Flavivirus*). It was first isolated in 1947 from a sentinel rhesus monkey during a yellow fever study in the Zika forest of Uganda.
Since its discovery, ZIKV circulation has been detected in Africa and Asia where it has caused sporadic human infections. In 2007 its emergence on Yap Island, Micronesia was reported, marking transmission of Zika virus outside Africa and Asia. Since 2013, ZIKV has been reported from French Polynesia, New Caledonia, Cook Islands, Easter Island (Chile), Samoa and Vanuatu, and in early 2015 it spread initially to Brazil and subsequently to additional countries of the Americas.

ZIKV is transmitted primarily through the bite of an infected Aedes species mosquito (*A. aegypti* and *A. albopictus*). However, there have been reports of less common transmission modes, such as blood transfusion, perinatal, and sexual contact. The incubation period of Zika virus disease is not known precisely, but is likely to be a few days.
It is estimated that only one in five people infected with ZIKV develop signs or symptoms. Clinical manifestations of ZIKV infection are described as very similar to those of Dengue virus (DENV) and Chikungunya virus (CHIKV) infections, but usually milder.
The most common clinical signs and symptoms are maculopapular rash, low grade fever, arthralgia, myalgia, headache and conjunctivitis. Less frequently reported are oedema, sore throat, cough, vomiting, and haematospermia.
Human infections with ZIKV are usually mild and self-limiting and the symptoms usually resolve spontaneously after 3–7 days; arthralgia may persist for up to 1 month. In rare cases, after a Zika virus infection a Guillain-Barré syndrome (GBS), a disorder of the peripheral nerves, can probably occur. A correlation between a Zika virus infection in pregnancy and congenital brain malformations is now considered likely.

Species	**Disease**	**Symptoms (e.g.)**	**Transmission route**
Zika Virus (ZIKV)	Zika fever	Fever, headache, retro-orbital pain, conjunctivitis, maculopapular rash, myalgias, arthralgias	Primary mode of transmission via bite of infected *Aedes* mosquitos

The presence of pathogen or infection may be identified by

Isolation in cell culture
PCR
Serology: Detection of antibodies by IF, ELISA
Plaque Reduction Neutralization Test (PRNT)

2. INTENDED USE

The Zika Virus IgM µ-capture ELISA is intended for the qualitative determination of IgM class antibodies against Zika virus in human serum or plasma (citrate, heparin).

3. 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-class antibodies to bind the corresponding antibodies of the sample. After washing the wells to remove all unbound sample material, horseradish peroxidase (HRP) labelled antigen is added. This antigen-conjugate binds to the captured specific IgM antibodies. In a second washing step unbound conjugate is removed. 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.

4. MATERIALS

4.1. Reagents supplied

Zika Virus Coated Microplate (IgM): 12 break-apart 8-well snap-off strips coated with anti-human IgM-class antibodies; in resealable aluminium foil.

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 Solution: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.

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.

Zika Virus Conjugate: 1 bottle containing 15 ml of peroxidase labelled Zika virus antigen; coloured red; ready to use; black cap.

TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap; < 5 % NMP.

Zika Virus IgM Positive Control: 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.

Zika Virus IgM Cut-off Control: 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.

Zika Virus IgM Negative Control: 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

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

4.3. 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

5. STABILITY AND STORAGE

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

6. REAGENT PREPARATION

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

6.1. Coated Microplate

The break-apart snap-off strips are coated with anti-human IgM-class antibodies. 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.

6.2. 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.

6.3. 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.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. For CSF please contact info@novateinbio.com. 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 s amples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. 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.

8. ASSAY PROCEDURE

8.1. 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 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the 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 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at 37 ± 1 °C.** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. 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.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. 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.

9. RESULTS

9.1. 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.

9.2. 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 [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

10. 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 NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
# 1	24	1.012	4.02
# 2	24	0.488	3.03
# 3	24	0.431	1.84
Interassay	**n**	**Mean (NTU)**	**CV (%)**
# 1	12	25.22	10.74
# 2	12	11.42	5.29
# 3	12	6.77	8.83

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 98.62 % (95% confidence interval: 95.11% - 99.83%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100 % (95% confidence interval: 71.51% - 100.0%).

10.4. 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.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal significant evidence of false-positive results due to cross-reactions. However, in endemic areas, double infection as well as past infection with other flaviviruses should be considered.

11. LIMITATIONS OF THE PROCEDURE

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

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. 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 patient samples.
- Only for in-vitro diagnostic 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.
- 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 patient 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.

12.1. 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.

13. ORDERING INFORMATION

Prod. No.: NB-E400926M Zika Virus IgM µ-capture ELISA (96 Determinations)

SUMMARY OF TEST PROCEDURE

SCHEME OF THE ASSAY

Zika 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	-	100 µl	-	-	-
Cut-off Control	-	-	100 µl	-	-
Positive Control	-	-	-	100 µl	-
Sample (diluted 1+100)	-	-	-	-	100 µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37 °C** Wash each well three times with 300 µl of Washing Buffer					
Conjugate	-	100 µl	100 µl	100 µl	100 µl
Incubate for 30 min at 37°C Do not expose to direct sunlight Wash each well three times with 300 µl of Washing Buffer					
TMB Substrate solution	100 µl	100 µl	100 µl	100 µl	100 µl
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					