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ab213327 anti-Zika virus IgM µ-capture ELISA kit

For the qualitative determination of IgM antibodies to Zika virus in human serum or plasma (citrate, heparin).

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's anti-Zika virus IgM µ-capture in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab213327) is designed for the qualitative determination of IgM antibodies to Zika virus in human serum or plasma (citrate, heparin).

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 Zika virus antigen is added. This antigen-conjugate binds to the captured specific IgM antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is 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 specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color.

Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

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.

2. Protocol Summary

Prepare all reagents, samples, controls and standards as instructed.

Add samples, standards and controls to wells used.

Add prepared labeled HRP-Conjugate to each well. Incubate at 37°C.

After washing, add TMB substrate solution to each well. Incubate at room temperature.

Add Stop Solution to each well. Read immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition (before preparation)	Storage Condition (after preparation)
Anti-Human IgM coated Microplate (12 x 8 wells)	96 wells	4°C	4°C
IgM Sample Diluent	100 mL	4°C	4°C
Stop Solution	15 mL	4°C	4°C
20X Wash Buffer Concentrate	50 mL	4°C	4°C
Zika Virus Conjugate	15 mL	4°C	4°C
TMB Substrate Solution	15 mL	4°C	4°C
Zika Virus IgM Positive Control	2 mL	4°C	4°C
Zika Virus IgM Cut-off Control	3 mL	4°C	4°C
Zika Virus IgM Negative Control	2 mL	4°C	4°C
Cover foil	1 Unit	4°C	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 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 µL and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

8. Technical Hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.
- Briefly centrifuge small vials at low speed prior to opening.

9.1 Zika Virus IgM Microplate:

The ready to use 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 aluminum foil along with the desiccant supplied and stored at 4□C.

9.2 IgM Sample Diluent:

1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; colored green; ready to use and stored at 4□C.

9.3 Stop Solution:

1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use and stored at 4□C.

9.4 20X Wash Buffer Concentrate:

1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M); pH 7.2 ± 0.2; for washing the wells and stored at 4□C. Dilute Washing Solution 1 + 19; e. g. 10 mL Washing Solution + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature.

9.5 Zika Virus Conjugate

1 bottle containing 15 mL of peroxidase labelled Zika Virus antigen; coloured red; ready to use and stored at 4□C.

9.6 TMB Substrate Solution

1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB) < 0.1%; ready to use and stored at 4□C away from the light. The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.□

9.7 Zika Virus IgM Positive Control:

1 bottle containing 2 mL control (human serum or plasma); colored yellow; ready to use and stored at 4□C.

9.8 Zika Virus IgM Cut-off Control:

1 bottle containing 3 mL control (human serum or plasma); colored yellow; ready to use and stored at 4□C.

9.9 Zika Virus IgM Negative Control:

1 bottle containing 2 mL control (human serum or plasma); colored yellow; ready to use and stored at 4□C.

9.10 Cover foil

Ready to use and stored at 4□C.

10. Sample Preparation

- Use human serum or plasma (citrate or heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 4°C; otherwise they should be aliquoted and stored deep-frozen (-70°C to -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.
- Before assaying, all samples should be diluted 1/100 with IgM 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.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

11. Assay Procedure

- 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 Solution from 300 µL to 350 µL to avoid washing effects.
- Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established 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°C ± 1 °C.

11.1 Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.

11.2 Cover wells with the foil supplied in the kit.

11.3 Incubate for 1 hour ± 5 min at 37°C ± 1°C.

11.4 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 Solution. Avoid overflows from the reaction wells. The interval between washing und aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step.

ΔNote: Washing is important! Insufficient washing results in poor precision and false results.

11.5 Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.

11.6 Incubate for 30 min at room temperature. Do not expose to direct sunlight.

11.7 Repeat step 11.4.

11.8 Dispense 100 µL TMB Substrate Solution into all wells.

11.9 Incubate for exactly 15 min at room temperature in the dark. A blue color occurs due to an enzymatic reaction.

11.10 Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate, thereby a color change from blue to yellow occurs.

11.11 Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

12. Calculations

Adjust the ELISA Microwell Plate Reader to zero using the Substrate Blank.

If the ELISA Microwell Plate Reader cannot be adjusted to zero using the Substrate Blank, subtract the 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 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.

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

Run Validation Criteria

- **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.

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

Results in Abcam Units [AU]

Sample (mean) absorbance value x 10 = [Abcam Units = AU]
Cut-off

Example: $\frac{1.591 \times 10}{0.43} = 37$ AU (Units)

Interpretation of Results

Result	Value
Cut- off	10 AU
Positive	> 11 AU
Equivocal	9 – 11 AU
Negative	< 9 AU

13. Typical Sample Values

SENSITIVITY –

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100 % (9/9).

Example of Sensitivity and Specificity in patient samples

The purpose of this study was to determine the efficiency of the assay to discriminate between positive and negative clinical samples.

To evaluate the performance of the Abcam anti-Zika Virus IgM µ-capture ELISA, internal studies were conducted with well-defined samples.

Samples from newborns and immunocompromised individuals were excluded from the study as in these patient's serological data only have limited value.

122 samples

- Defined ZIKV samples: 13 samples
- Defined negative samples: 43 pregnancy samples (healthy women, Germany)

  4 blood bank samples (Frankfurt, Germany)
- Cross reactivity panel: 62 samples (incl. United States, Uganda, Guadeloupe, New Caledonia)

		Positive	Negative	Total
Abcam anti-Zika Virus IgM µ-capture ELISA Kit	Positive	9	2	11
	Negative	0	110	110
	Total	9	112	121

(Equivocal results were not included in the calculations)

SPECIFICITY –

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 98.2% 110/112).

AGREEMENT –

98.3% (119/121)

PRECISION –

Intra-assay

Sample	N	CV (%)
1	24	2.7
2	24	1.65
3	24	1.92

Inter-assay

Sample	N	CV (%)
1	12	8.02
2	12	5.59
3	12	9.17

INTERFERENCES –

- Increased concentrations of possible interference materials such as bilirubin, triglycerides and hemoglobin may interfere with immunoassays creating false-negative or false-positive results.
- In order to investigate this topic a literature research in combination with investigation of nearly 100 parameters was performed.
- Different Abcam ELISA kits were used including assays for the detection of different antibody isotypes (IgA, IgG, IgM, IgG + IgM) to bacteria, viruses, fungi, parasites and worms as well as an antigen ELISA for the detection of TSH.
- Defined positive resp. negative or equivocal samples were used.
- A certain amount of the potentially interfering substance was added to each sample. The final concentration of each substance was in a pathological range as also described by competitors (10 mg/mL hemoglobin, 0.5 mg/mL bilirubin and 5 mg/mL triglycerides). The results obtained with the sample with added "interfering substance" should be 60-140 % of the result of the untreated sample in order to fulfil the specifications.

- The internal specifications of 60-140 % were always fulfilled.
- Interferences with hemolytic, lipemic or icteric samples were not observed up to a concentration of 10 mg/mL hemoglobin, 0.5 mg/mL bilirubin and 5 mg/mL triglycerides.

14. Species Reactivity

- A panel of 62 specimens from patients with confirmed diseases other than Zika virus (ZIKV) infection was tested to establish the analytical specificity of Abcam's anti-Zika Virus IgM µ-capture ELISA kit.
- The specimens were from patients infected with pathogens that may cause similar signs and symptoms to those observed for ZIKV (e.g. DENV, CHIKV) or from individuals with diseases or conditions that have the potential for cross-reactivity such as other members of the family Flaviviridae (WNV, TBEV, YFV) or pathogens involved in polyclonal B-cell activation (Epstein-Barr virus, EBV).

Pathogen/Disease Type	Number of Samples	Positive Result
Dengue virus (DENV)	12	0/12
West Nile virus (WNV)	20	1/20
Tick borne encephalitis virus (TBEV)	3	0/3
Chikungunya virus (CHIKV)	5	0/5
Yellow Fever virus (YFV)	12	1/12
Epstein-Barr virus	4	0/4
Rheumatoid Factor	5	0/5
ANA	1	0/1
Total	62	2/62

- Investigation of a specimen panel with antibody activities to potentially cross-reacting parameters revealed only minimal cross-reactivity to West Nile virus (WNV) and Yellow Fever virus (YFV) specimens.
- In endemic areas, double infection as well as past infection with other flaviviruses should be considered.

15. Quick Assay Procedure

ΔNOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

14.1. Diluted samples 1/100 with IgM Sample Diluent.

14.2. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.

14.3. Cover wells with the foil supplied in the kit.

14.4. Incubate for 1 hour ± 5 min at 37°C ± 1°C.

14.5. Aspirate and wash 3 times with 300 µL of Washing Solution. Tap strips on tissue paper prior to the next step.

14.6. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.

14.7. Incubate for 30 min at room temperature. Do not expose to direct sunlight.

14.8. Repeat wash step.

14.9. Dispense 100 µL TMB Substrate Solution into all wells.

14.10. Incubate for 15 min at room temperature in the dark.

14.11. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate.

14.12. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

Please contact our Technical Support team for more information.

16. Troubleshooting

Problem	Cause	Solution
Low signal	Incubation time to short	Repeat
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

17. Notes

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