Zika Virus IgM ELISA

Product code: Prototype

Instructions for Use

The NativeAntigen COMPANY

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About the Native Antigen Company

The Native Antigen Company is accredited to ISO9001:2015 standard. We are proud to by powered by 100% renewable energy as part of our commitment to protect the environment. Should you have any comments or feedback, please get in touch, we love to hear from our customers.

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1. INTENDED USE

The Native Antigen Company (NAC) Zika Virus IgM ELISA assay is designed for the detection of Zika-specific IgM antibodies in human serum. It is minimally cross-reactive with antibodies to Dengue virus (a closely-related flavivirus), and so can be used to distinguish human anti-Zika antibodies from other flavivirus and infectious disease antibodies in the early stages of Zika infection. Use of the assay is intended for detection of IgM in patients with Zika infection.

This product is currently a prototype, it is not to be used as a diagnostic.

2. BACKGROUND INFORMATION

Zika virus (ZIKV) is a member of the virus family Flaviviridae and the genus *Flavivirus*, transmitted by Aedes mosquitoes, such as *A. aegypti* and *A. albopictus*. Its name comes from the Zika Forest of Uganda, where the virus was first isolated in 1947. Zika virus is related to Dengue, Yellow Fever, Japanese Encephalitis, and West Nile viruses, and many antibodies (e.g. those produced as a response to infection) cross-react between these viruses. This cross-reaction causes severe problems in identifying individuals seropostive for Zika as the much wider spread background of Dengue infections cause many false positives in most standard serological ELISAs.

NAC's Zika virus IgM antibody capture ELISA uses a double antigen blocking assay using in-house manufactured antigens to enhance sensitivity and virtually exclude cross reactions. It is therefore suitable for the accurate detection of serum IgM antibodies to Zika virus, indicating recent exposure. This can be used as a diagnostic assay, but as this is currently in prototype form, it should not be used as such until full registration has been achieved.

3. MATERIALS SUPPLIED

Component	Amount	Storage temp
Pre-coated microtitre plate	TBC	TBC
Negative control (NC) (Lyophilised)	TBC	TBC
Positive control (PC) (Lyophilised)	TBC	TBC
Differential control (DC) (Lyophilised)	TBC	TBC
Blocking Reagent	TBC	TBC
Detection Reagent 2	TBC	TBC
Sample diluent	TBC	TBC
Reagent 1 diluent	TBC	TBC
25X Wash buffer	TBC	TBC
TMB substrate	TBC	TBC
Stop solution	TBC	TBC
Plate sealers	TBC	TBC

4. STORAGE CONDITIONS

a. To be confirmed

5. MATERIALS REQUIRED BUT NOT SUPPLIED

a. Microtitre plate reader capable of absorbance measurement at 450nm and preferably capable of dual wavelength correction between 600nm and 650nm
b. Deionized water
c. Precision pipettes covering the range 5µl to 1ml
d. Microtitre plate shaker capable of 800 orbital revolutions per minute (rpm)
e. Vortex mixer
f. Disposable microcentrifuge tubes

6. WARNINGS AND PRECAUTIONS

This kit is fcurrently at prototype stage. DO NOT USE FOR DIAGNOSTIC PURPOSES. Observe the following precautions:

a. Laboratory coats and other PPE as appropriate should be worn when using the kit
b. Hands should be washed thoroughly after use
c. If handling potentially hazardous samples, use appropriate containment precautions
d. Clean up spillages and decontaminate any affected surfaces
e. Take care not to generate aerosols
f. Ventilate the work area adequately
g. Dispose of any waste and materials in accordance with local regulations

Materials of a biological origin should be handled as if capable of causing infection, using appropriate precautions and good clinical laboratory practices, even if samples have been tested for infectious agents or from a source considered safe. Decontaminate with an appropriate disinfectant and store and dispose of such materials and containers according to local regulations.

WARNING: POTENTIAL CHEMICAL HAZARD

Stop solution is 1M Hydrochloric acid, which is corrosive, causing severe damage to eyes, skin and mucous membranes, therefore wear hand, face and eye protection when handling this material. This kit contains Proclin 950 and TMB, which can be irritants to skin and mucous membranes at high concentrations. They are supplied in dilute form, and as such should present a minimal risk. In case of direct contact with any of these materials, wash the area thoroughly with water and seek medical advice.

7. SAMPLE DETAILS

Human-derived samples should be handled as follows:

- Routine precautions for venipuncture should be followed when collecting blood samples.
- Allow time for the blood to fully clot.
- Within 3 hours of collection, centrifuge to separate serum, and collect at least 500µl of sample without cells in a securely capped sample tube.
- Samples not tested on day of preparation must be frozen at -20°C or colder for storage or shipment.
- This assay has only been evaluated on human serum.
- Avoid repeated freeze/thaw cycles, no more than 3 times.

8. ASSAY PROTOCOL

(Total assay time 4 hours & 15 minutes)

1. Reconstitute the controls using 500ul of sample diluent. Leave it for 10 minutes at RT and vortex before use. Dilute the controls further 1:101 (5ul + 500ul) in sample buffer provided, mix thoroughly before adding it to the microwells.
2. Add 100ul of Controls / serum sample dilution (1:101) in each well and incubate the plate for 1 hour at 18-22°C (Seal the plate)
3. Aspirate the plate contents, taking any safety precautions as required by sample type. Fill each well with 300ul of 1x wash buffer, perform 3 wash/empty cycles, then invert the plate onto an absorbent paper and pat away any excess wash buffer.
4. Wash the plate 3 times using 1X wash buffer
5. Blocking reagent: Reconstitute each freeze-dried blocking reagent vial with 3mls of diluent buffer, allow it to stand for 10 minutes. Gently mix before addition to the plate.
6. Add 100ul of Blocking reagent per well and incubate the plate for 1 hour at 18-22°C (Seal the plate)
7. Wash the plate 3 times using 1X wash buffer
8. Detection reagent: Reconstitute each freeze-dried detection reagent vial with 3mls of diluent buffer, allow it to stand for 10 minutes.

Gently mix before addition to the plate.

9. Add 100ul of Detection reagent in each well and incubate the plate for 1 hour at 18-22°C (Seal the plate)
10. Wash the plate 5 times using 1X wash buffer
11. Add 100ul of Detection reagent 2 in each well and incubate the plate for 1 hour at 18-22°C (Seal the plate)
12. Wash the plate 5 times 1X wash buffer
13. Add 100ul of TMB substrate in every used well and incubate for 15 minutes at 18-22°C in the dark (Seal the plate using a fresh plate sealer)
14. Add 100ul of Stop solution per well, gently mix and read the plate at 450/620nm within 10 minutes of stopping the reaction

9. RESULT VALIDITY

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

- Negative control : The mean absorbance value of negative controls must be less than the mean differential control value
- Differential control : The mean absorbance value should be between TBD and TBD inclusive
- Positive control : The mean absorbance value of positive controls must be higher than the mean differential control value

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

10. RESULT CALCULATIONS AND INTERPRETATION

Calculate the Ratio Value (RV) for each sample by dividing the mean Optical Density (OD) value of each sample by the mean OD of the differential control:

Ratio Value = Mean OD of sample / Mean OD of differential control

The results should then be interpreted as follows:

Ratio	Interpretation
< 0.9	Negative for Zika IgM
≥ 0.9 but ≤ 1.0	Indeterminate
> 1.0	Positive for Zika IgM

Indeterminate samples are to be considered positive for flavivirus IgM, but may either be low levels of Zika IgM, or very high levels of related flavivirus IgM most likely Dengue.

11. SENSITIVITY AND SPECIFICITY

The following clinical samples of disease state sera/plasma were tested in this assay using the standard protocol. Of the Zika negative sera, 47 were positive for Dengue IgM, 13 for WNV IgM, 11 for CHIKV IgM and 3 healthy donors.

Zika IgM positive patient sera				
Total	Positive	Indeterminate	Negative	Sensitivity
23	22	0	1	95.7%
Zika IgM negative patient sera				
Total	Positive	Indeterminate	Negative	Specificity
74	68	3	3	95.9%

For the purposes of the above calculations, indeterminate results were taken as negative.

12. INTERFERING SUBSTANCES

Serum samples were spiked with common interfering substances and evaluated in comparison with un-spiked material.

Substance	Amount	Result
Haemoglobin	2 mg/mL	TBC
Bilirubin	1 mg/mL	TBC
Rheumatoid factor	54 IU/ml	TBC
Triolein	45.5 mg/ml	TBC

Problems with lipaemia can arise in non-fresh freeze/thawed samples. To avoid false positives, centrifuge the sample and avoid the lipid layer and pellet.

13. TROUBLESHOOTING

The following table may be used to troubleshoot the assay.

Issue	Possible 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
	Increase dilution factor of sample	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

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