Zika Virus NS1 Capture ELISA

Product code: ELS61231

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 NS1 Capture ELISA kit is designed for the quantitative measurement of Zika virus NS1 (ZIKV NS1) in whole blood, serum/plasma, urine and culture fluids.

This product is FOR RESEARCH USE ONLY, and is not for use in diagnostic procedures.

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 virus, Yellow Fever, Japanese Encephalitis, and West Nile viruses, and antibodies (e.g. those produced as a response to infection) may cross-react between these viruses.

The viral genome is expressed as a single large polypeptide, and NS1 is an early marker of viral replication. It has been reported in human serum (Bosch et al Sci Transl Med. 9 (409) (2017)).

3. PRINCIPLE OF THE TEST

The NAC ZIKV NS1 Capture ELISA uses two specific monoclonal antibodies to capture and detect ZIKV NS1. These antibodies are specific for ZIKV NS1, and do not cross-react with Dengue virus, WNV, JEV, YFV, Usutu or TBEV NS1.

Samples containing the target analyte are pre-diluted in a diluent designed to reduce interfering factors and enhance binding. The diluted samples are then incubated in microtitre plate wells coated with stabilised capture antibody. After incubation and washing, a second specific antibody coupled to biotin is added to detect the bound NS1, and then subsequently streptavidin-HRP is used to probe the biotinylated antibody. Finally TMB is added to give a coloured product in proportion to the amount of HRP bound and the reaction stopped with acid solution.

Absorbance of the wells is measured at 450nm and a reference reading may also be taken between 620-650 nm. Standards supplied with the kit are used to plot a standard curve of OD vs. concentration, from which the concentrations of the samples may be calculated.

4. MATERIALS SUPPLIED

- 96 well microtitre plate with stabilised anti-Zika virus NS1 coated wells in a resealable pouch with desiccant
- Kit standards, 5 bottles lyophilised
- Zero standard (**BLUE**), one bottle, 30ml including 0.5% Proclin 950
- Wash Buffer 25X, one bottle, 100ml
- Antibody-biotin conjugate (**PINK**) ready to use, one bottle, 12ml including 0.5% Proclin 950
- Streptavidin-HRP conjugate ready to use, one bottle, 12ml including 0.5% Proclin 950
- TMB ready to use, one bottle, 12ml
- 1M Hydrochloric Acid, one bottle, 12ml
- Self-adhesive sealing film, x6

5. STORAGE CONDITIONS

a. Store the un-opened kit at +2 to +8°C until the expiry date on the outer box label
b. After reconstitution in zero standard the kit standards must be stored at =< -20°C until the expiry date shown on the outer kit label
c. The 1 x wash buffer is stable for 1 week at room temperature (18-28°C) in a sealed container.
d. After opening the plate pouch, wells must be stored in the sealed pouch with desiccant sachet at 2 to 8°C until the expiry date shown on the outer kit label
e. Do not use the kit after the expiry date on the outer packaging
f. Where multiple lots of kits have been purchased, do not combine components between kit lots, even if within shelf life

6. MATERIALS REQUIRED BUT NOT SUPPLIED

a. Microtitre plate reader capable of absorbance measurement at 450 nm and preferably capable of dual wavelength correction between 600 and 650 nm
b. Deionized water
c. Precision pipettes covering the range 50 to 500ul
d. Microtitre plate shaker capable of 500–700 orbital revolutions per minute (rpm)
e. Microtitre plate washer
f. Vortex mixer
g. Disposable glass or polystyrene tubes

7. WARNINGS AND PRECAUTIONS

This kit is for Research Use Only. DO NOT USE FOR DIAGNOSTIC PURPOSES. Observe the following precautions:

a. Do not eat, drink, smoke or apply cosmetics where the assay kit is being used
b. Do not mouth pipette
c. Laboratory coats and other PPE as appropriate should be worn when using the kit
d. Hands should be washed thoroughly after use
e. If handling potentially hazardous samples, work in a spill tray
f. Clean up spillages and decontaminate any affected surfaces
g. Take care not to generate aerosols
h. Ventilate the work area adequately
i. Dispose of any waste and materials in accordance with local regulations

There is no additional risk to using the kit routinely with blood and body fluid derived samples when using this procedure. However 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 contact direct contact with any of these materials, wash the area thoroughly with water and seek medical advice.

8. SAMPLE DETAILS

The assay may be used to quantitatively measure ZIKV NS1 in serum, urine and culture medium. The assay may also be used to detect the presence of ZVNS1 in whole blood and plasma collected in Lithium Heparin and EDTA K3 anticoagulants, but may not quantitate. Human derived samples should be handled as follows:

a. Routine precautions for venepuncture should be followed when collection blood samples.
b. If collecting serum, allow time for the blood to fully clot before separating.
c. If collecting plasma, ensure the blood is fully mixed with the anticoagulants according to the blood tube manufacturer's instructions.
d. No more than 24 hours after collection centrifuge the serum or plasma to separate. Within 3 hours of separation collect at least 500µl of sample without cells in a securely capped sample tube.
e. Samples not tested on day of preparation must be frozen at -20°C or colder for storage or shipment.
f. Other sample types should be clarified by centrifugation on the day of collection, and stored at -20°C or colder for storage or shipment.

- Avoid repeated freezing and thawing of samples.
- Avoid storing samples for long periods at 2-8°C or higher.
- Avoid assaying lipaemic or haemolysed samples.

9. TEST PROTOCOL

Reagent preparation
Allow all reagents to warm to ambient temperature for 30 minutes before using the kit. Mix liquid reagents by gentle inversion or on a rocking roller mixer prior to use.

Wash buffer: Dilute the 25x wash buffer 1 in 25 with purified lab grade water to make 1x wash buffer.

Microtitre strips: Select the number of strips required for the standards and samples to be run. Store all unused strips sealed in the foil pouch with the supplied silica gel sachet to protect them from moisture.

Standards: The standards must be reconstituted in **BLUE** zero standard, using the volume indicated on the label. After addition allow the standard to stand for 5 minutes, then mix gently for a further 10 minutes. The standards are then ready to use, and must not be diluted.

Samples: Samples that show a precipitate should be centrifuged to remove the debris before use. Dilute all samples a minimum of 1 in 10 (50µl plus 450µl diluent suggested) in **BLUE** zero standard, ensuring that the diluted samples are thoroughly mixed. If a sample gives a higher signal than the top standard then it should be diluted and re-tested to ensure it falls within the standard range. Note that very high samples may require substantial pre-dilution.

Assay protocol

1. Prepare a plate plan to indicate the position of standards and samples on the plate. All standards and samples must be run in duplicate.
2. Add 100µl volumes of all standards (including **BLUE** zero standard) and samples to the wells as indicated by the plate plan. Cover the plate with self-adhesive sealing film and incubate the plate on a shaker set to 800rpm for 2 hours at ambient (18-28°C) temperature.
3. Aspirate the plate contents, taking any safety precautions as required by sample type. Fill each well with 300µl of 1x wash buffer before aspirating without a delay or soak time. Perform 3 wash/empty cycles, then invert the plate onto paper towel and pat away excess wash buffer.
4. Add 100µl **PINK** Antibody-Biotin reagent to every well. Cover the plate with the sealing film and incubate with shaking for 1 hour at ambient (18-28°C) temperature.
5. Aspirate and wash the plate following the method described in step 3.
6. Add 100µl streptavidin-HRP reagent to every used well. Cover the plate with the sealing film and incubate with shaking for 30 minutes at ambient (18-28°C) temperature.
7. Aspirate and wash the plate following the method described in step 3. Use 6 cycles of washing and aspiration instead of 3.
8. Add 100µl volumes of TMB substrate to every used well. Cover the plate with a new sealing film and incubate with shaking for 15 minutes at ambient (18-28°C) temperature. Be aware that direct sunlight may cause a reduction in colour development; therefore the plate should be protected from strong sunlight.
9. Add 100µl of stop solution to every well. Read the plate at 450nm, reference at 620nm (605nm-650nm) within 15min of stopping.

10. RESULTS

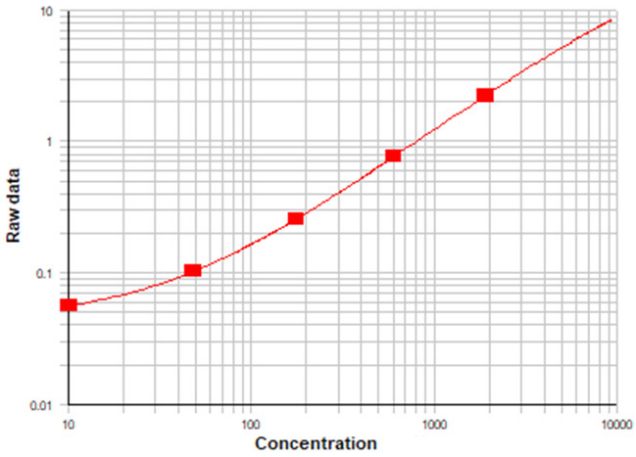
1. Calculate the mean 450nm absorbance for each standard or sample, minus the reference wavelength value if available. Use the zero standard OD value as a blank.
2. Using a 5 parameter logistic curve-fit, calculate the sample values. If a 5 parameter fit is not available from the data reduction software then a 2nd order polynomial fit may be used instead. Use of a 4PL fit is not recommended.
3. Any sample reading higher than the highest standard should be appropriately diluted using **BLUE** zero standard and re-assayed.
4. Any sample reading lower than the bottom standard should be reported as such.
5. Multiply the sample value by any dilution factor, as required.

11. EXAMPLE DATA

Typical standard curve, data obtained using OD450nm – Note: this data is intended as an example only, and must not be used as a standard curve to calculate sample values.

ZIKV NS1 pg/ml	Replicate A	Replicate B
1906	2.367	2.349
603	0.798	0.822
177.5	0.266	0.266
48.3	0.100	0.107
10.2	0.054	0.054
0	0.042	0.040

The above data fitted using a 5 parameter curve fit in Magellan software for Tecan F50 plate reader:



12. CALIBRATION

The assay has been calibrated against NAC ZIKVSU-NS1 Suriname antigen spiked into zero standard/sample diluent.

13. ANALYTICAL SENSITIVITY

Sensitivity of the kit was estimated from 20 replicates of the Zero standard/ sample diluent run as samples in the assay. A number equivalent to 3 standard deviations added to the mean OD value for those replicates was calculated and read back from the standard curve on 4 occasions for each of 3 separate kit lots shared across 2 operators. Typical sensitivity of the kit is lower than 5pg/ml.

14. INTER-ASSAY REPRODUCIBILITY

3 control samples were run on 36 occasions across 3 kit lots and 3 operators.

Control samples	pg/ml	CV%
High	1176.0	6.3
Mid	340.5	9.6
Low	47.8	11.1

15. INTRA-ASSAY REPRODUCIBILITY

14 samples were run in sets of 10 replicates across 3 kit batches on a single occasion. Average intra-assay variability was 1.9%.

16. ASSAY DRIFT

Sets of samples near the top, middle and bottom of the standard curve were run in duplicates added to the plate at 5 minute intervals in 3 kit lots. Using least squares regression, the time at which the concentration would vary by +/- 10% of the intercept value was calculated. In all cases the time at which the concentration would reach +/- 10% was greater than 25 minutes.

17. SPIKE RECOVERY

Recombinant Zikavirus NS1 Suriname strain (NAC product ZIKVSU-NS1-100 lot 17110116) gave an average 98.6% recovery when spiked to 150pg/ml into zero standard/sample diluent.

Recombinant Zikavirus NS1 Uganda strain (NAC product ZIKV-NS1-100 lot 17082910) gave an average 84.4% recovery when spiked to 150pg/ml into zero standard/sample diluent.

Samples of different materials were spiked with NAC recombinant ZIKV NS1 of either Uganda or Surinam strain as shown and the recoveries calculated from the equivalent spike into zero standard/sample diluent.

Sample type	Uganda Recovery %	Suriname Recovery %
Urine pool stored frozen	86.8	88.0
Urine single donor fresh	90.7	92.2
Spent culture medium	98.6	97.3
Fresh culture medium	91.5	92.7
Single donor serum	89.5	92.4
Whole blood lithium heparin	ND	68.1
Plasma lithium Heparin	68.7	72.3
Whole blood EDTA K3	ND	60.3
Plasma EDTA K3	56.7	53.7

18. LINEARITY

5 samples were diluted 1 in 20, 1 in 40 and 1 in 100 and values compared to the standard 1 in 10 dilution. Average recovery was 113.1%.

19. CROSS-REACTIVITY

Zero standard/sample diluent was spiked with NS1 from various related flaviviruses to 10,000 pg/ml

Virus	pg/ml	% CR
Dengue 1 virus	44.2	0.4
Dengue 2 virus	5.1	0.1
Dengue 3 virus	2.1	0.0
Dengue 4 virus	1.9	0.0
West Nile Virus	0.0	0.0
Japanese Encephalitis virus	6.9	0.1
Yellow Fever Virus	3.1	0.0

20. INTERFERING SUBSTANCES

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

Interferent	Concentration mg/ml	Unspiked sample value pg/ml	Spiked sample value pg/ml	Difference %
Haemoglobin	2mg/ml	1142.4	1238.8	105.7
		324.6	405.5	124.9
	1mg/ml	1142.4	1256.8	107.2
		324.6	371.9	114.6
	0.5mg/ml	1142.4	1217.5	103.8
		324.6	352.765	108.7
Bilirubin	1mg/ml	1142.4	1229.8	104.9
		324.6	356.505	109.8
Triolein	45.5mg/ml	1142.4	1108.5	94.5
		324.6	322.48	99.3
Rheumatoid factor	108 IU/ml	1142.4	1152.9	98.3
		324.6	344.315	106.1

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