Product Manual

# Zika NS1 Protein ELISA Kit

**Catalog Number**

**VPK-5161** **96 assays**

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures**

CELL BIOLABS, INC.
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## Introduction

Zika virus is a single-stranded RNA virus that causes Zika virus disease and is a member of the genus Flavivirus, which includes West Nile virus, Dengue virus, and Yellow Fever virus. Zika is mainly transmitted by the female *Aedes aegypti* mosquito. The Zika virus RNA genome is translated into a single polyprotein which encodes capsid (C), membrane (M), and 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The nonstructural proteins aid in replicating and packaging of the RNA genome as well as in inhibiting various host cell pathways.

Cell Biolabs' Zika NS1 Protein ELISA Kit is an enzyme immunoassay designed to measure Zika (strain MR 766) NS1 Protein from cell or tissue samples. It provides sufficient reagents for up to 96 tests in a 96-well plate including standard curve and unknown samples. Detection sensitivity is 20 pg/mL.

## Related Products

1. VPK-5145: SARS-Cov-2 Nucleocapsid ELISA Kit
2. VPK-5154: West Nile Virus Envelope Protein ELISA Kit
3. VPK-107: Quick Titer™ Lentivirus Titer Kit (Lentivirus-associated p24 ELISA)
4. VPK-109: QuickTiter™ Adenovirus Titer Immunoassay Kit
5. VPK-112: QuickTiter™ Lentivirus Quantitation Kit
6. VPK-120: QuickTiter™ Retrovirus Quantitation Kit
7. VPK-145: QuickTiter™ AAV Quantitation Kit

## Kit Components

### Box 1 (shipped at room temperature)

1. Anti-Zika NS1 Protein Antibody Coated Plate (Part No. 51611B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Zika NS1 Protein Antibody (1000X) (Part No. 51612C): One 10 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. Triton X-100 Solution (Part No. 310805): One 15 mL bottle containing 5% Triton X-100 in tris buffered saline (TBS).
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

**Box 2 (shipped on blue ice packs)**

1. Zika NS1 Protein Standard (Part No. 51613D): One 50 µL vial of 125 ng/mL recombinant Zika NS1 protein.

## Materials Not Supplied

1. Microcentrifuge
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

## Storage

Upon receipt, aliquot and store the Zika NS1 Protein Standard and the Biotinylated Anti-Zika NS1 Protein Antibody at -80ºC. Avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

## Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Zika NS1 Protein Antibody and Streptavidin Enzyme Conjugate: Immediately before use, dilute the Biotinylated Anti-Zika NS1 Protein Antibody and the Streptavidin Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

## Preparation of Standard Curve

1. Prepare a dilution series of Zika NS1 Protein standard in the concentration range of 0 to 1250 pg/mL into Assay Diluent (Table 1).

| Standard Tubes | Zika NS1 Protein Standard (µL) | Assay Diluent (µL) | Zika NS1 Protein (pg/mL) |
|---|---|---|---|
| 1 | 5 | 495 | 1250 |
| 2 | 250 of Tube #1 | 250 | 625 |
| 3 | 250 of Tube #2 | 250 | 313 |
| 4 | 250 of Tube #3 | 250 | 156 |
| 5 | 250 of Tube #4 | 250 | 78.1 |
| 6 | 250 of Tube #5 | 250 | 39.1 |
| 7 | 250 of Tube #6 | 250 | 19.5 |
| 8 | 0 | 250 | 0 |

**Table 1. Preparation of Zika NS1 Protein Standard**

2. Transfer 225µL of each dilution (Standard Tubes 1-8) to a microcentrifuge tube containing 25 µL of Triton X-100 Solution. Perform the assay as described in Assay Instructions.

## Preparation and Inactivation of Samples

1. (Optional) Dilute Zika samples in culture medium or assay diluent as needed. For unknown samples we recommend several dilutions for each sample. Include culture medium as a negative control.
2. Transfer 225 µL of each sample to a microcentrifuge tube containing 25 µL of Triton X-100 Solution. Vortex well.
3. Incubate 60 minutes at 60°C.

## Assay Protocol

1. Add 100 µL of Zika NS1 Protein unknown sample or standard to the Anti-Zika NS1 Protein Antibody Coated Plate. Each Zika NS1 Protein unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at room temperature for 1 hour on an orbital shaker.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-Zika NS1 Protein antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   *Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.*
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

## Example of Results

The following figures demonstrate typical results with the Zika NS1 Protein ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

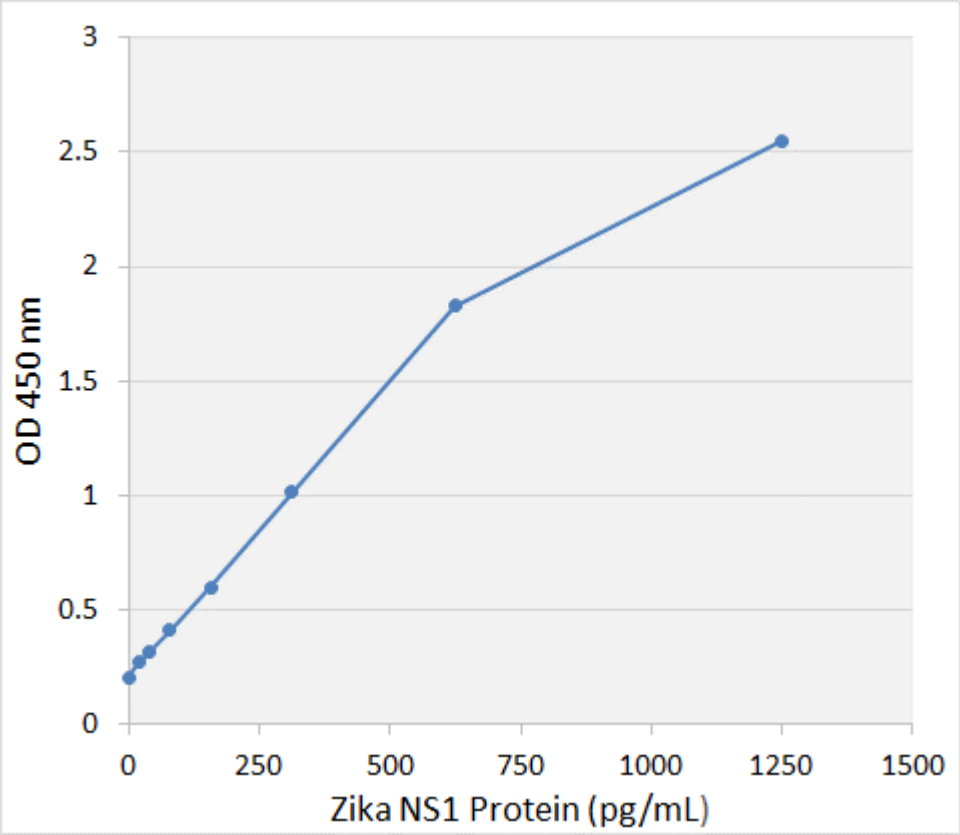


**Figure 1: Zika NS1 Protein ELISA Kit Standard Curve.**

## References


1. Sirohi D and Kuhn RJ (2017) *J. Infec. Dis.*, **S10**:S935-S944
2. Kazmi SS, Ali W, Bibi N, and Nouroz F (2020) *J. Biol Res (Thessalon)* **27**:1-11.
3. Higenfeld R (2016) *EMBO J*. 35: 2631-2633
4. Guo M, Hui L, Nie Y, Tefsen B and Wu Y (2021) *Sci. China Life Sci*. **64**:709–719


## Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED

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## Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com