ELISA PRODUCT INFORMATION & MANUAL

Zika Virus (SPH2015) NS1 ELISA Kit (Colorimetric)

NBP2-80364

Sample Insert for Reference Only

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

BACKGROUND

Zika virus NS1 antigen is one of seven non-structural proteins. NS1 is involved in RNA replicaiton. The possible effects of NS1 on hosts include: localization to host cell surface and secreted extracellularly, modelates signalling of innate immune system, has possible damages to platelets and endothelial cells through anti-NS1 antibodies.

INTENDED USE

The kit has been verified by high purity, recombinant Zika Virus (SPH2015) NS1 Protein.

The use of this kit for natural samples need be validated by the end user due to the complexity of natural targets and unpredictable interference.

PRINCIPLE OF THE ASSAY

The principle of this ELISA kit is based on the solid phase sandwich enzyme immunoassay technique. A monoclonal antibody specific for Zika Virus (SPH2015) NS1 has been pre-coated onto well plate strips. Standards and samples are added to the wells and Zika Virus (SPH2015) NS1 present in the sample is bound by the immobilized antibody. After incubation the wells are washed and a horseradish peroxidase conjugated anti-Zika Virus (SPH2015) NS1 antibody is added, producing an
antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody a TMB substrate solution is loaded and color develops in proportion to the amount of Zika Virus (SPH2015) NS1 bound. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm (See schematics below).

Capture Antibody   Analyte   HRP-conjugated Detection Antibody
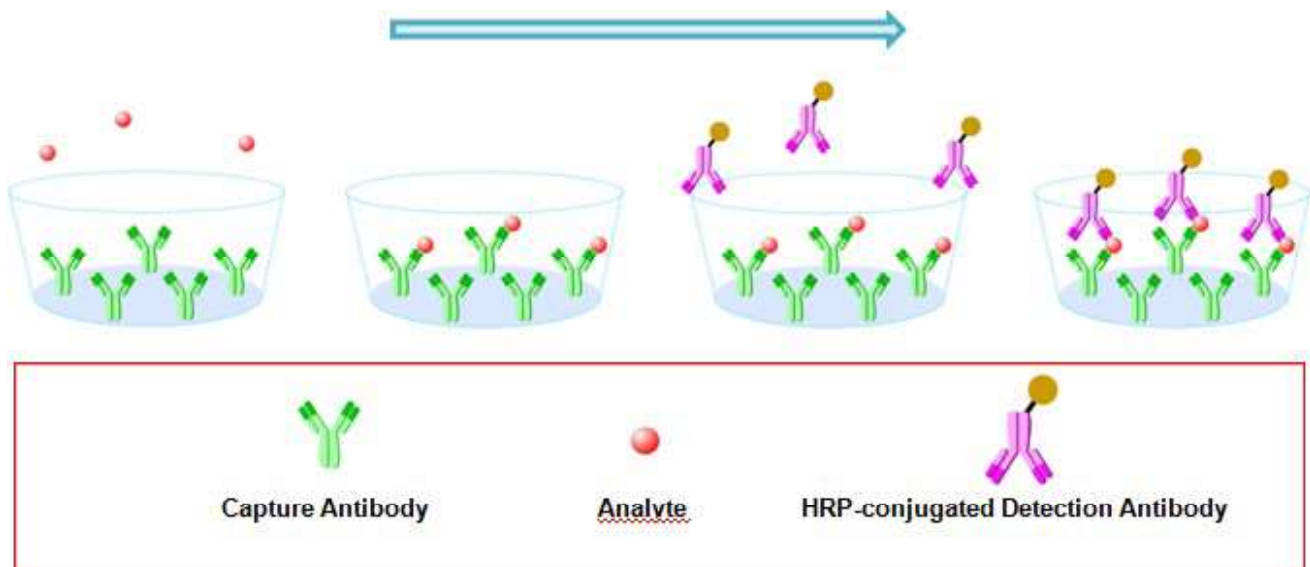
MATERIALS PROVIDED

Zika Virus (SPH2015) NS1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with mouse mAb antibody against Zika Virus (SPH2015) NS1.

Zika Virus (SPH2015) NS1 Detection Antibody - 0.2 mg/mL of mouse mAb antibody against Zika Virus (SPH2015) NS1 conjugated to horseradish peroxidase (HRP) with preservatives.

Zika Virus (SPH2015) NS1 Standard - Recombinant Zika Virus (SPH2015) NS1 in a buffer with preservatives, lyophilized. The amount of standard is lot specific and indicated on the label of standard vial.

Wash Buffer Concentrate - 25 mL of a 20-fold concentrated solution of buffered surfactant with preservatives.

Dilution Buffer Concentrate - 8 mL of a 20-fold concentrated dilution buffer with preservatives.

Color Reagent A - 13 mL of stabilized hydrogen peroxide.

Color Reagent B - 13 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution - 8 mL of 2 N sulfuric acid.

STORAGE

Unopened Kit	Store at 2 - 8℃ and the kit is stable for 6 months upon receipt.	
Opened/ Reconstituted Reagents	Diluted Wash Buffer	Stored for up to 1 week at 2 - 8℃
	Diluted Dilution Buffer	
	Conjugate	Stored for up to 1 month at 2 - 8℃
	Stop Solution	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	After reconstitution, store for up to 1 month at -80℃. The reconstituted standards should be aliquoted and **avoid repeated freeze-thaw cycles**.
	Microplate Wells	Return unused strips to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal. Stored for up to 1 month at 2 - 8℃

OTHER SUPPLIES REQUIRED

·Microplate reader capable of measuring absorbance at 450 nm

·Pipettes and pipette tips

·Deionized or distilled water

·Multi -channel pipette, squirt bottle, manifold dispenser, or automated microplate washer

·500 mL graduated cylinder

·Tubes for standard dilution

·Well plate cover or seals

PRECAUTIONS

1. This kit is **for research use only** and is not for use in diagnostic or therapeutic procedures.

2. The kit should not be used beyond the expiration date.

3. Do not mix reagents from different lots.

4. The kit is designed and tested to detect the specific targets and samples shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

SAFETY INSTRUCTIONS

5. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.

6. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.

7. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

TECHINICAL TIPS

8. Bring all reagents and samples to room temperature before use.

9. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.

10. A standard curve should be generated for each set of sample assayed. DO NOT USE the standard curves from other plates or other days.

11. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.

12. Read the absorbance of each well within 20 minutes after adding the stop solution.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.

Wash Buffer - Prepare 1× wash buffer by adding 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 400 mL of Wash Buffer.

Dilution Buffer - Prepare 1× dilution buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer.

Detection Antibody - Centrifuge at 10,000 x g for 20 seconds. Dilute to **work concentration** of 0.5 μg/mL in Dilution Buffer before use.

Substrate Solution **-** Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well. **Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.**

Zika virus (strain Zika SPH2015) ZIKV-NS1 Standard - Reconstitute the Zika virus (strain Zika SPH2015) ZIKV-NS1 Standard with 1 mL of Dilution Buffer to make stock solution. Shake the vial gently until the lyophilized powder totally dissolved (**Do not turn the vial upside down**). Mix the standard to ensure complete reconstitution prior to making dilutions.

Prepare serially diluted standards as described in the following step:

Pipette 1000 μL of Dilution Buffer into the 5000 pg/mL tube. Pipette 500 μL of Dilution Buffer into the remaining tubes. Use the stock solution to produce a dilution series as the following figure. Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). **Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.**

The following graph is only for demonstration purposes. The concentration of stock solution is lot specific and need be calculated with the actual amount of standard labeled on the standard vial.

248,000 pg/mL   5000 pg/mL   2500 pg/mL   1250 pg/mL   625 pg/mL   312.5 pg/mL   156.25 pg/mL   78.13 pg/mL
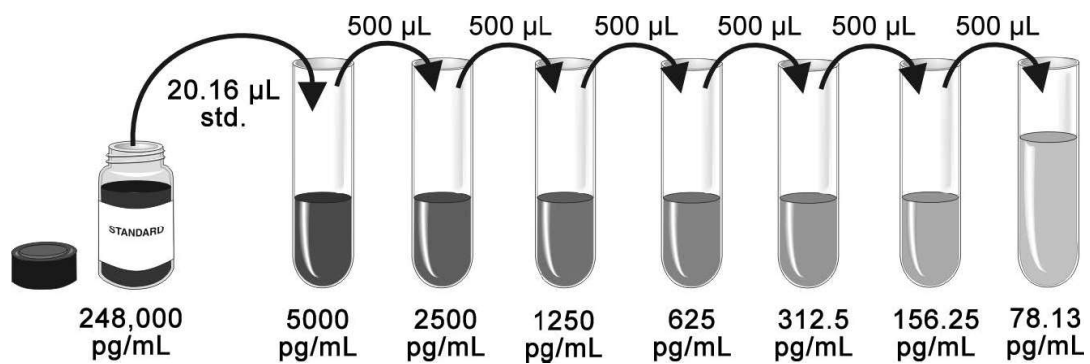
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Remove unused microplate strips from the plate frame, return them to the foil pouchcontaining the desiccant pack, and reseal.

3. Wash each well three times with Wash Buffer (300 μL/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. **Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.**

4. Add 100 μL of each serially diluted protein standard or test sample per well including a zero standard. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover/seal the plate and incubate for 2 hours at room temperature.

5. Repeat the aspiration/wash as in Step 3.

6. Add 100 μL of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in Step 3.

8. Add 200 μL of Substrate Solution to each well. Incubate for 20 minutes at room temperature**. Protect from light.**

9. Add 50 μL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. **Determine the optical density of each well within 20 minutes**, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.) .

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Most graphing software can help make the curve and a four parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

Concentration (pg/mL)	Zero standard subtracted OD
0	0
78.13	0.037
156.25	0.091
312.5	0.188
625	0.348
1250	0.685
2500	1.291
5000	2.392
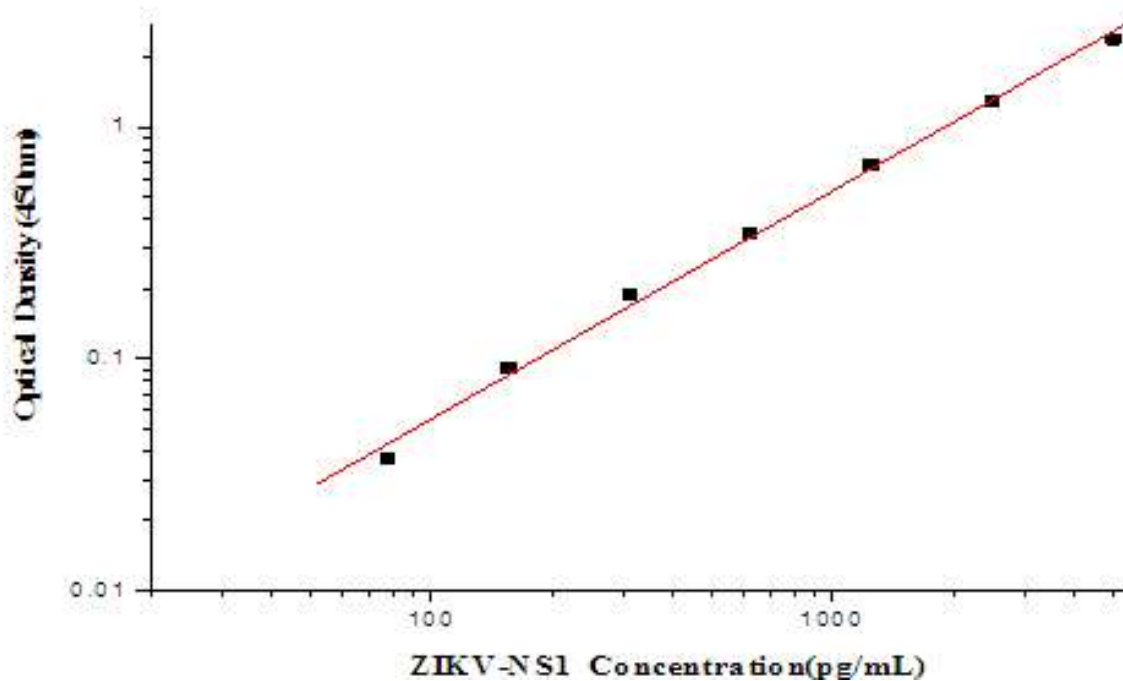
PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in five separate assays to assess inter-assay precision.

	Intra -assay Precision			Inter -assay Precision		
Sample	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	975	2004	4161	1013	2020	3994
SD	39.32	88.78	159.99	52.03	59.02	153.37
CV (%)	4.0%	4.4%	3.8%	5.1%	2.9%	3.8%

RECOVERY

The recovery of Zika virus (strain Zika SPH2015) ZIKV-NS1 spiked to different levels throughout the range of the assay in related matrices was evaluated.

Sample	Average % Recovery	Range
Cell culture supernates (n=3)	100	95-104%

LINEARITY

		Cell culture supernates
1:2	recovery of detected	101%
1:4	recovery of detected	99%
1:8	recovery of detected	95%
1:16	recovery of detected	104%

SENSITIVITY

The minimum detectable dose (MDD) of Zika virus (strain Zika SPH2015) ZIKV-NS1 is typically less than 58.39 pg/mL. The MDD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified HEK 293-expressed recombinant Zika virus (strain Zika SPH2015) ZIKV-NS1.

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃. The reconstituted standards should be aliquoted and avoid repeated freeze-thaw cycles.
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	Color Reagent should be clear and colorless prior to addition to wells	Color Reagent should be clear and colorless prior to addition to wells
	Use clean tubes and pipettes tips	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

ASSAY SUMMARY

Prepare reagents and standard as instructed.

↓

Remove excess microplate strips, Wash each well three times

↓

Add 100 μL standard and sample to each well within 15 minutes.

↓ ← Incubate 2 hours at RT.

Aspirate and wash 3 times.

↓

Add 100 μL Detection antibody to each well.

Incubate 1 hour at RT. → ↓

Aspirate and wash 3 times.

↓

Add 200 μL Substrate Solution to each well.

Incubate 20 minutes at RT. →
Protect from light. ↓

Add 50 μL Stop Solution to each well.

↓

Read at 450nm within 20 minutes and calculate the concentration of target antigen.