Version 25-09/25

User's Manual

West Nile virus NS1 Antigen ELISA Kit

REF **DEIAY10297**

Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

West Nile virus NS1 Antigen ELISA Kit is intended for the quantitative analysis of NS1(WNV) concentrations in cell culture supernatants,serum, plasma and other biological samples within the range of 1.56-100ng/mL in a sandwich ELISA format.

Reagents And Materials Provided

1. **Capture Antibody Coated 96 well microtiter plate:** 1 plate; store at 2-8°C
2. **Standard Protein:** 20μL of 50μg/mL recombinant NS1(WNV); store at -80°C, avoid repeated freezing and thawing (No more than 5 times)
3. **100× Detection Antibody:** 120μL of HRP-anti NS1(WNV) monoclonal antibody; store at -20°C
4. **Sample Diluent Solution:** 50mL; store at 2-8°C
5. **Enzyme Conjugate Diluent Solution:** 12mL; store at 2-8°C
6. **20× WashBuffer:** 50mL; store at 2-8°C
7. **TMB Solution:** 12mL; store at 2-8°C
8. **Stop Solution:** 7mL; store at 2-8°C

Materials Required But Not Supplied

1. Microplate reader capable of reading absorbance at 450 nm.
2. Automated plate washer (optional).
3. Pipettes and pipette tips. Multichannel pipettes are recommended for a large numbers of samples.
4. Deionized or distilled water.
5. Test tubes for dilution.
6. 37°C incubator.

Storage

Refer to Reagents and Materials Provided to store the components of the ELISA kit

Plate Preparation

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 100 ng/mL	Std 100 ng/mL	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
B	Std 50 ng/mL	Std 50 ng/mL	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
C	Std 25 ng/mL	Std 25 ng/mL	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
D	Std 12.5 ng/mL	Std 12.5 ng/mL	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
E	Std 6.25 ng/mL	Std 6.25 ng/mL	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
F	Std 3.125 ng/mL	Std 3.125 ng/mL	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38
G	Std 1.56 ng/mL	Std 1.56 ng/mL	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31	Sample 39	Sample 39
H	Std 0 ng/mL	Std 0 ng/mL	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32	Sample 40	Sample 40

Assay Procedure

1. Standard/Sample: Dilute standard with Sample Diluent Solution to eight concentrations (100ng/mL, 50ng/mL, 25ng/mL, 12.5ng/mL, 6.25ng/mL,3.125ng/mL, 1.56ng/mL, and 0ng/mL).

   The sample was diluted 1:10 with Sample Diluent Solution. For this, 20 uL of sample is added to 180 uL of diluent solution.

   Add 100μL of Standard/Sample to each well in duplicate. Incubate at 37°C for 1 hour.
2. Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with 350 uL of diluted Wash Solution (20× Wash Solution was diluted 20 times), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of Five washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.
3. Dilute 100μL of Detection Antibody with 9900μL of Detection Ab Diluent Solution to prepare a detection solution. Add 100μL of the detection solution into each well. Incubate at 37°C for 1 hour.
4. Aspirate and wash plate 5 times.
5. Add 100μL TMB Solution into each well. Incubate at 37°C for 30 minutes.
6. Add 50μL of Stop Solution to each well.
7. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450nm.

Calculation

Plotting the Standard Curve and Determining the Sample Concentration: Using computer reduction software, plot absorbance (linear y-axis) versus concentration (log x-axis) for standards and fit the data with a four-parameter logistic equation. Using the equation of the line, calculate the concentration of analyte in each sample, making sure to correct for any sample dilution.

Interpretation Of Results

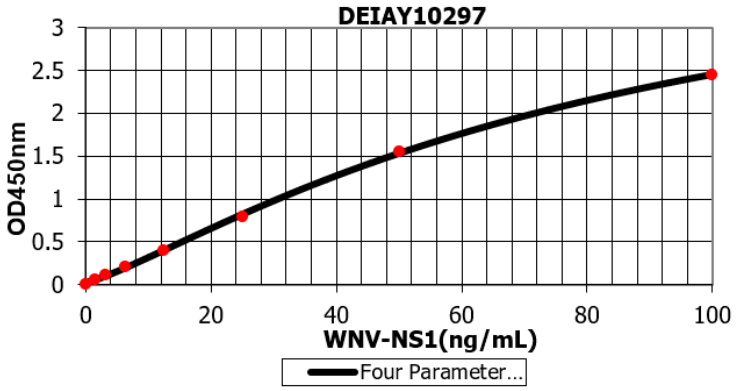
Cut-off value = OD value of Blank Control × 2

Positive: OD value of sample ≥Cut-off value, indicating a positive NS1(WNV) antigen in the sample

Negative: OD value of sample < Cut-off value, indicating a negative NS1(WNV) antigen in the sample.

Typical Standard Curve

STD. (ng/mL)	OD450	
100	2.4583	2.4447
50	1.5193	1.5846
25	0.7788	0.8061
12.5	0.3967	0.4002
6.25	0.2074	0.2202
3.125	0.1145	0.0978
1.5625	0.0493	0.0534
0	0.0045	0.0051
R^2	0.999	



Precision

Conc.	Inter CV	Intra CV
50ng/mL	2.93%	9.70%
15ng/mL	0.95%	5.80%
1.56ng/mL	6.61%	7.28%

Detection Range

1.56-100 ng/mL

Sensitivity

Limit of Quantitation: 1.56 ng/mL

Limit of Detection: 0.43 ng/mL

Recovery

Serum	Recovery
50ng/mL	81%
25ng/mL	83%
10ng/mL	90%

Precautions

1. The reagents involved in the experiment were diluted with ultra-pure water to working concentration (1×).
2. Allow the reagents to reach room temperature (20-30°C) prior to testing.

3. For research use only.

Tel: 1-631-624-4882 (USA)
Tel: 44-161-818-6441 (Europe)
Fax: 1-631-938-8221
Email: info@creative-diagnostics.com