Version 28-04/24

User's Manual

Anti-West Nile Virus ELISA Kit (IgG)

REF **DEIA-BY005**

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.

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PRODUCT INFORMATION

Intended Use

The Anti-West Nile Virus ELISA (IgG) test kit provides a semiquantitative or quantitative assay for human antibodies of the IgG class against West Nile virus in serum or plasma.

Principles of Testing

The test kit contains microtiter strips each with 8 break-off reagent wells coated with West Nile virus antigens. In the first reaction step, diluted samples are incubated in the wells. In the case of positive samples, specific IgG antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Reagents And Materials Provided

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL2
4. Calibrator 3 2 RU/ml, (IgG, human), ready for use	light red	1 x 2.0 ml	CAL3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	
14. Protective foil	---	2 pieces	

Materials Required But Not Supplied

1. Microplate reader
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Storage

The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Specimen Collection And Preparation

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Samples are diluted 1:101 in sample buffer. For example: dilute 10 µl serum in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.

Plate Preparation

The pipetting protocol for microtiter strips 1-4 is an example for the semiquantitative analysis of 24 samples (P 1 to P 24). The pipetting protocol for microtiter strips 7-10 is an example for the quantitative analysis of 24 samples (P 1 to P 24). The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample. The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage. Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 2	P 6	P 14	P 22			C 1	P 4	P 12	P 20		
B	pos.	P 7	P 15	P 23			C 2	P 5	P 13	P 21		
C	neg.	P 8	P 16	P 24			C 3	P 6	P 14	P 22		
D	P 1	P 9	P 17				pos.	P 7	P 15	P 23		
E	P 2	P 10	P 18				neg.	P 8	P 16	P 24		
F	P 3	P 11	P 19				P 1	P 9	P 17			
G	P 4	P 12	P 20				P 2	P 10	P 18			
H	P 5	P 13	P 21				P 3	P 11	P 19			

Reagent Preparation

All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below. The thermostat adjusted ELISA incubator must be set at 37°C +/- 1°C.

- Coated wells: Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag). Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- Calibrators and controls: Ready for use. The reagents must be mixed thoroughly before use. - Enzyme conjugate: Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- Sample buffer: Ready for use.

- Wash buffer: The wash buffer is a 10× concentrate. If crystallisation occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water). For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water. The working strength diluted wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- Chromogen/substrate solution: Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- Stop solution: Ready for use.

Assay Procedure

For semiquantative analysis incubate calibrator 2 along with the positive and negative controls and patient samples. For quantitative analysis incubate calibrators 1, 2 and 3 along with the positive and negative controls and patient samples.

1. Sample incubation: Transfer 100 µl of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation, follow the instrument manufacturer's recommendations with regard to microwell plate sealing. Incubate 60 minutes at 37°C ± 1°C.
2. Washing: Manual: Remove the protective foil and empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash. Automatic: Remove the protective foil and empty the wells and subsequently wash 3 times with 450 µl of working strength wash buffer. Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer. Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to false high extinction values. Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.
3. Conjugate incubation: Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. When using an automated microplate processor for incubation, follow the instrument manufacturer´s recommendations with regard to microwell plate sealing. Incubate 30 minutes at room temperature (+18°C to +25°C)
4. Washing: Empty the wells. Wash as described above.

5. Substrate incubation: Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for 15 minutes at room temperature (+18°C to +25°C) (protect from direct sunlight).
6. Stopping the reaction: Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.
7. Measurement: Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Interpretation Of Results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2. Calculate the ratio according the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

CD recommends interpreting results as follows:

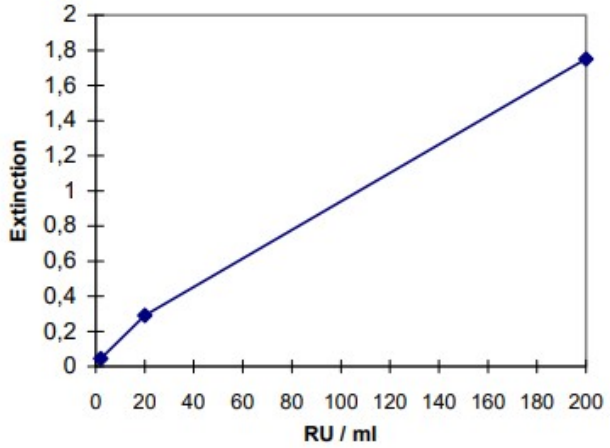
Ratio <0.8: negative

Ratio ≥0.8 to <1.1: borderline

Ratio ≥1.1: positive

In cases of borderline test results, an additional sample should be taken 7 days later and re-tested in parallel with the first sample. The results of both samples allow proper evaluation of titer changes.

Quantitative: The standard curve from which the concentration of antibodies in the samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use "point-to-point" plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in samples.



If the extinction of a serum sample lies above the value of calibrator 1 (200 RU/ml). The result should be given as ">200 RU/ml". It is recommended that the sample be re-tested at a dilution of 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range of non-infected persons (cut-off value) recommended by CD is 20 relative units (RU)/ml. CD recommends interpreting results as follows:

<16 RU/ml: negative

≥16 to <22 RU/ml: borderline

≥22 RU/ml: positive

Evaluation information: For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For the interpretation of borderline results an investigation using further tests (e.g. avidity determination of antibody class IgG) can be helpful.

Performance Characteristics

Calibration: As no quantificated international reference serum exists for antibodies against West Nile virus, the calibration is performed in relative units (RU). For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated. The activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibration sera are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The antigen source is a recombinant, detergent-extracted glycoprotein E of WNV from the membrane fraction of human cells.

Detection Limit

Detection limit: The lower detection limit is defined as the mean value of an analyte-free samples plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-West Nile Virus ELISA (IgG) is 0.4 RU/ml.

Specificity

Cross reactivities to other flaviviruses cannot be excluded. They were recognized with anti-TBE positive and anti-Dengue virus positive samples.

Antibodies against	n	Anti-West Nile Virus ELISA (IgG)
Adenovirus	12	0%
Chlamydia pneumoniae	12	0%
CMV	12	0%
EBV-CA	12	0%
Helicobacter pylori	12	0%
HSV-1	12	0%
Influenza virus A	12	0%
Influenza virus B	12	0%
Measles virus	12	0%
Mumps virus	12	0%
Mycoplasma pneumoniae	12	0%
Parainfluenza virus Pool	12	0%
RSV	12	0%
Rubella virus	12	0%
Toxoplasma gondii	9	0%
VZV	12	0%

Linearity

The linearity of the Anti-West Nile Virus ELISA (IgG) was determined by assaying 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was> 0.95. The Anti-West Nile Virus ELISA (IgG) is linear at least in the tested concentration range (10 RU/ml to 160 RU/ml).

Reproducibility

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Serum	Mean value (RU/ml)	CV (%)
1	101	6.1
2	104	3.3
3	149	2.7

Inter-assay variation, n = 4 x 6		
Serum	Mean value (RU/ml)	CV (%)
1	103	5.1
2	120	4.9
3	171	4.2

Interferences

Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Precautions

1. Waste disposal: Samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.
2. The controls used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid skin contact.