DRG

DRG® West Nile IgG (EIA-4519)

CE

USA: RUO

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Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

The West Nile IgG Test for exposure to West Nile Virus (WNV) is an ELISA assay system for the detection of antibodies in human serum to WNV derived recombinant antigen (WNRA) (1-3). It is not intended to screen blood or blood components.

2 PRINCIPLE OF THE TEST

The West Nile IgG ELISA consists of one enzymatically amplified "two-step" sandwich-type immunoassays.

In this assay, the microtitration wells are incubated with standards, controls or unknown serum samples. The serum samples may be directly mixed with sample dilution buffer added in the wells (also see note below). After washing, the wells are treated with an antibody specific for human IgG and labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate.

Note: Depending on the strength of antibody response, sera can be diluted in a diluent provided in the kit.

An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbances of the WNRA and the control wells accurately determines whether antibodies to WNV are present. A set of positive and negative samples is provided as internal controls in order to monitor the integrity of the kit components.

3 MATERIALS SUPPLIED

The West Nile IgG ELISA contains sufficient reagents for one plate of 96 wells (12 x 8 strips) each. Each kit contains the following reagents:

IgG Assay Specific Materials:

1. **Coated Microtiter Strips** for **WN Human IgG**
   Strip holder with zip lock foil pouch, containing 96 polystyrene microtiter wells (12 x 8 wells) coated with monoclonal antibody bound to recombinant WN antigen. Store at 2-8ºC until ready to use.
   Note: The WNRA and NCA are already bound to plates**.**
2. **Sample Dilution Buffer for IgG** One bottle, 25 ml,
   for sample dilution.
   Store at 2-8°C until ready to use.
3. **WN IgG Positive Control** One vial, 50 µL.
   The positive control will aid in monitoring the integrity of the kit .
   Store at 2-8°C until ready to use for up to 7 days.. Quick spin the vial briefly before use to collect the content at the bottom.
   Note: For long-term storage, serum can be further aliquoted in a smaller volume and stored at -20 to -70°C.

4. **WN IgG Negative Control** One vial, 50 µL.
   The negative control will aid in monitoring the integrity of the kit as well.
   Store at 2-8°C until ready to use for up to 7 days. Quick spin the vial briefly before use to collect the content at the bottom.
   Note: For long-term storage, serum can be further aliquoted in a smaller volume and stored at -20 to -20°C.
5. **Ready to Use Enzyme Conjugate-HRP for WN IgG** One bottle, 6 mL
   of a pre-diluted goat anti-human IgG conjugate to be used as is in the procedure below.
   Store at 2-8°C until ready to use.
   The conjugate should be kept in a light - protected bottle at all times as provided.
6. **10X Wash Buffer** One bottle, 120 mL,
   to be used in all the washing steps of this procedure.
   Store 10X Wash Buffer at 2-8°C until ready to use.
7. ***En*Wash** One bottle, 20 mL,
   to be used in between the washing steps after the addition of enzyme conjugate-HRP of this procedure.
   Store *En*Wash Buffer at 2-8°C until ready to use.
8. **Liquid TMB Substrate** One bottle, 9 mL.
   Store at 2-8ºC until ready to use.
   The substrate should be kept in a light -protected bottle at all times as provided.
9. **Stop Solution** One bottle, 6 mL
   to be used to stop the reaction.
   Store at 2-8°C until ready to use.
   Caution: strong acid–wear protective gloves, mask and safety glasses. Dispose of all materials according to safety rules and regulations.

NOTE:
All reagents and controls must be allowed to reach room temperature (~25°C) and mixed thoroughly by gentle inversion prior to use. Always practice sterile and aseptic techniques at every step. For example, open all reagents in a sterile hood to avoid contamination with airborne bacteria to maintain shelf life.

4 MATERIALS REQUIRED BUT NOT SUPPLIED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Biological or High-Grade Water
- Vacuum Pump
- Humidified Incubator or Water Bath
- Single-Channel and Multi-Channel Pipettors

5 PRECAUTIONS

- A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
- Do not mix various lots of any kit component within an individual assay.

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- Do not use any component beyond the expiration date shown on its label.
- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
- Some reagents may form a slight precipitate, mix gently before use.
- Incomplete washing will adversely affect the outcome and assay precision.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the TMB solution.
- Avoid microbial contamination of reagents, especially of the conjugate concentrate and the conjugate diluent. Avoid contamination of the TMB Substrate Solution with the Enzyme Conjugate-HRP.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic materials are being handled.
- Do not pipet by mouth.
- Use a clean disposable pipette tip for each reagent, Standard, Control or specimen.
- Cover working area with disposable absorbent paper.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit may contain reagents made with human serum or plasma. The serum or plasma used has been heat inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

CHEMICAL HAZARD:
Material Safety Data Sheets (MSDS) are available for all components of this kit. Review all appropriate MSDS before performing this assay. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable MSDS for appropriate treatment.

6 SPECIMEN COLLECTION AND PREPARATION

- Human serum must be used with this assay. Whole blood or plasma cannot be tested directly.
- Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
- Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods.
- Serum should be used and the usual precautions for venipuncture should be observed. The samples may be stored at 2-8°C for up to 7 days, or frozen at -20°C or lower for up to 30 days. To maintain long-term longevity of the serum, store at -70°C. Avoid repeated freezing and thawing of samples.
- Do not use hemolyzed or lipemic samples.
- Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

7 TEST PROCEDURE

Bring all kit reagents and specimens **to room temperature (~25°C) before use**. Thoroughly mix the reagents and samples before use by gentle inversion.

Note: For long-term storage, all serum, including the experimental, cannot be repeatedly thawed and frozen. Sera should be further aliquoted in a smaller volume and stored at –70°C.

7.1 Preparation of Reagents

1X Wash Buffer

Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water.

To prepare a 1X wash buffer solution, mix 120 ml 10X wash buffer with 1080 ml distilled (or deionized) water and rinse out any crystals. Swirl until well mixed and all crystals are dissolved.

After diluting to 1X, store at room temperature for a maximum of four months.
Note: Discard the 1X Wash Buffer if you see any microbial growth.

Microtitration Wells

Select the number of coated wells required for the assay. The remaining unused wells should be covered and placed back quickly into the pouch and stored at 2-8°C until ready to use or expiration.

7.2 Assay Procedure

Allow all reagents to reach room temperature (~25°C) and mix thoroughly by gentle inversion before use. Standards, controls and unknown serum to be tested should be assayed in duplicate.

IgG Assay:

1. Mark the microtitration strips to be used.
   Note that the West Nile Antigens (WNRA) and control antigens (NCA) are already bound to the plate in the same arrangement as described in the following table.

West Nile Antigen	Strip #1	Strip #2
A	WNRA	WNRA
B	WNRA	WNRA
C	WNRA	WNRA
D	WNRA	WNRA
E	NCA	NCA
F	NCA	NCA
G	NCA	NCA
H	NCA	NCA

2. In a small, polypropylene tube prepare a 1:300 dilution of the serum sample(s), positive and negative controls in Sample Dilution Buffer for IgG.

3. Add 50 µL of each diluted serum sample to each well. An exemplary arrangement for one serum sample using only one microtiter strip is shown below.
   Note: Samples and controls are to be assayed in WNRA and NCA coated wells.

	Strip #1	Strip #2
	Serum Sample	
A	IgG N	Test Sample #1
B	IgG N	Test Sample #1
C	IgG P	Test Sample #2
D	IgG P	Test Sample #2
E	IgG P	Test Sample #2
F	IgG P	Test Sample #2
G	IgG N	Test Sample #1
H	IgG N	Test Sample #1

4. Cover the strips and incubate for one hour at 37°C in an incubator.
5. Wash the strips six (6) times with the 1X Wash Buffer using an automatic plate washer (300 µl per well per cycle).
6. Add 50 µL of "Ready to Use Enzyme Conjugate-HRP" to each well.
7. Cover the strips and incubate for one hour at 37°C in an incubator.
8. After the incubation, wash the strips six (6) times with the 1X Wash Buffer using an automatic plate washer.
9. Add 150 µL per well of EnWash and incubate for 5 minutes at room temperature (~25°C).
10. After the incubation, wash the strips six (6) times with the 1X Wash Buffer.
11. Add 75 µL of "Liquid TMB Substrate" to each well.
12. Cover the strips and incubate at room temperature (~25°C) in a dark container for 10 minutes.
13. Stop the reaction by adding 50 µL of "Stop Solution" to each well.
14. Read the plate immediately at 450 nm.
    Be sure the microplate reader does NOT subtract or normalize any blank values or wells

8 RESULTS

Results may vary from lot to lot. The results below are given strictly for guidance purposes only.

Applicable for spectrophotometric readings:

9 REFERENCES / LITERATURE

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6. Lanciotti RS, Roehrig JT, Deubel V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science. 1999;286(5448):2333-2337.
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