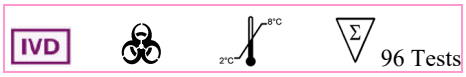
Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ West Nile IgG ELISA

Cat# 8400-25



Test	West Nile IgG
Method	Enzyme Linked Immunosorbent Assay
Principle	ELISA Two-Step Sandwich
Detection Range	Qualitative: Positive; Negative and Calibrator
Sample	4µL
Total Time	~ 180 min
Shelf Life	12 Months from the manufacturing date
Specificity	98.4%
Sensitivity	96.2%

INTENDED USE

The DAI West Nile IgG Test is an ELISA assay system for the detection of antibodies in human serum to West Nile Virus (WNV) derived recombinant antigen (WNRA) (1-3). This test is to aid in the diagnosis of human exposure to the West Nile Virus. It is not intended to screen blood or blood components. For Research Use Only. Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

Exposure to West Nile Virus causes a disease with a number of symptoms including encephalitis (4-7). West Nile Virus is becoming widespread and has been detected in over half of the 50 states. This test has been developed and refined using reagents produced by CDC. The DAI West Nile assay employs a recombinant antigen called WNRA, which can be used as a rapid serological marker for WNV infection. The WNRA protein is a recombinant antigen, which consists of a stretch of peptides from two WNV antigens.

TEST PRINCIPLE

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

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.

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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

The DAI 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. **Ready-to-use Coated WN IgG Microtiter Strips** Strip holder with plastic cover, 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** Two bottle, 25 ml, for serum sample dilution. Store at 2-8°C until ready to use.
3. **WN IgG Positive Control (IgG P)** One vial, 50 µL . The positive control will aid in monitoring the integrity of the kit as well. Store at 2-8°C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.
4. **WN IgG Negative Control (IgG N)** 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 . Quick spin the vial briefly before use to collect the content at the bottom.
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 of Wash Buffer to be used in all the washing steps of this procedure. Store 10X Wash Buffer at 2-8°C until ready to use.
7. **Wash Solution:** One bottle, 20 mL to be used in between the washing steps after the addition of enzyme conjugate-HRP of this procedure. Store Wash Solution 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 ℃) 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.*

Materials required but not provided

1. Microtitration plate reader capable of absorbance measurement at 450 nm
2. Biological or High-Grade Water
3. 37ºC incubator without CO_2 supply or humidification
4. Plate washer
5. Humidified Incubator or Water Bath
6. Single-Channel and Multi-Channel Pipettors
7. Timer

Specimen collection and preparation

1. Human serum must be used with this assay. Whole blood or plasma cannot be tested directly.
2. Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
3. Testing should be performed as soon as possible after collection. Do not leave Sera at room temperature for prolonged periods.
4. 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.
5. Do not use hemolyzed or lipemic samples.
6. Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use.
7. If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

ASSAY PROCEDURES

This kit has not been optimized by Diagnostic Automation Inc. for use with any particular automated ELISA processing system. Use with an automated ELISA processing system will require proper validation to ensure results are equivalent to the expectations described in this package insert. Modifications to the protocol of these systems and/or different volumes of reagents may be required.

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, sera cannot be repeatedly thawed and frozen. Sera should be further aliquoted in a smaller volume and stored at –70°C.

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 six months.
Note: Discard the 1X Wash Buffer if you see any microbial growth are observed.

- 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.

Assay Procedure:

Allow all reagents to reach room temperature (~25°C) and mix thoroughly by gentle inversion before use. Positive and negative controls should be assayed in duplicate. Test samples may be assayed in singlet.

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

	Strip #1	Strip #2
	Serum Sample	
A	Negative Control	Test Sample #1
B	Negative Control	Test Sample #2
C	Positive Control	Test Sample #3
D	Positive Control	Test Sample #4
E	Positive Control	Test Sample #4
F	Positive Control	Test Sample #3
G	Negative Control	Test Sample #2
H	Negative Control	Test Sample #1

4. Cover the strips and incubate for one hour at 37°C in a humidity chamber.
Note: Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO2 or other gas incubators. Do not place plates in contact with any wet substances such as wet paper towels, etc.
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 Wash buffer 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.

RESULTS

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

Data analysis:
For each sample and assay control, compute the average optical density (OD) of the two sample replicates with the WNRA, the two sample replicates with the NCA, and calculate the WNRA/NCA ratio (immune status ratio, or ISR).

Assay validity criteria:
The results in the table below must be obtained for discrimination capacity of the assay: Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

Factor	Tolerance
Mean Negative Control Reading (NC) WNRA OD	< 0.400
Mean Positive Control (PC) Reading WNRA OD	> 0.400
PC Immune Status Ratio (ISR)	> 3.000
NC Immune Status Ratio (ISR)	< 1.500

Example : Determination of assay validity
Calculate the mean Negative Control values with WNRA and with NCA:

Negative Control	(NC)	OD
	WNRA	NCA
No 1	0.135	0.126
No 2	0.125	0.110
Total	0.260	0.236

Averages (WNRA) = 0.260 ÷ 2 = 0.130
(NCA) = 0.236 ÷ 2 = 0.118
Calculate the WNRA/NCA ratio: 0.130 ÷ 0.118 = 1.10

Any Negative Control WNRA/NCA ratio greater than 1.500 indicates that the test procedure must be repeated.

Calculate the Positive Control values with WNRA and with the NCA.

Positive Control	(PC)	OD
	WNRA	NCA
No 1	0.635	0.190
No 2	0.655	0.178
Total	1.290	0.368

Averages (WNRA) = 1.290 ÷ 2 = 0.645
(NCA) = 0.368 ÷ 2 = 0.184
Calculate the WNRA/NCA ratio: 0.645÷ 0.184 = 3.5
Any Positive Control WNRA/NCA ratio less than 3.000 indicates that the test procedure must be repeated.

INTERPRETATION OF THE RESULTS

1. Samples with ISR > 3.0 should be presumed "Positive"
   Any "Positive" sample must be repeated to verify the result.
2. Samples with ISR <2.0 should be presumed "Negative".
3. Samples with ISR <3.0 but >2.0 should be considered "Equivocal"and should be repeated in triplicate.
4. ISR >2.0 arising from low optical densities in both the WNRA and NCA wells must be considered potential false positives.

ISR	Results	Interpretation
≤2.0	Negative	No detectable IgG antibody by the ELISA test
2.0-3.0	Equivocal	Need confirmatory test
≥3.0	Positive	Indicates presence of detectable IgG antibody. Recommend supplemental confirmatory testing.

False positive results have been reported to occur with certain conditions including, but not limited to, syphilis patients. Note also that WNRA/NCA ratios (ISR) >2.0 arising from low optical densities (OD) in both the WNRA and NCA wells must be considered potential false positives. See Example #1 in Exclusion Criteria.

Example #1: Low optical density samples:

Serum Sample	OD	
	WNRA	NCA
No 1	0.044	0.019
No 2	0.016	0.007
Total	0.060	0.026

Averages (WNRA) = 0.060 ÷ 2 = 0.030
(NCA) = 0.026 ÷ 2 = 0.013
Calculate the WNRA/NCA ratio: 0.030 ÷ 0.013 = 2.31

While the ISR is >2.0, this sample must be considered a potential false positive, due to the low optical densities and high relative standard deviations. This can occur when the plate reader subtracts relatively large values for the "blanks". It is important to not subtract the background from the OD readings.

PERFORMANCE CHARACTERISTICS

Sensitivity: In process.
Specificity: All well confirmed West Nile sera were positive by the DAI West Nile ELISA System. As a control, a number of normal sera and sera infected with unrelated disease were tested. All produced OD450 values which were below the cut-off value.
Interference: A small percentage of uncharacterized plasma samples containing rheumatoid factor were found to give ISR>3.0 (West Nile positive) in the IgG assay. Patients who have St. Louis or Japanese Encephalitis may have a positive result with the West Nile Assay.

LIMITATION OF PROCEDURE

1. Samples that generate high optical densities in the Antigen Control (non-WNRA) and thus ISR <3.0 may be false negatives. Diluting the sera further and re-testing may indicate the true ISR.
2. Since this is an indirect screening method, the presence of false positive and negative results must be considered.
3. All reactive samples must be evaluated by a confirmatory test.
4. The reagents supplied in this kit are optimized to measure WNRA reactive antibody levels in serum or plasma.
5. Repeated freezing and thawing of reagents supplied in the kit and of specimen must be avoided.
6. Hemolyzed and lipemic specimens may give false values and should not be used.

PRECAUTIONS

1. For research or export use only.

2. All human source material used in the preparation of controls has been heat-inactivated. However, all human controls and antigen should still be handled as potentially infectious material. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
3. 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.
4. Do not mix various lots of any kit component within an individual assay.
5. Do not use any component beyond the expiration date shown on its label.
6. Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
7. Unused microwells must be resealed immediately and stored in the presence of desiccant. Failure to do this may cause erroneous results.
8. Some reagents may form a slight precipitate, mix gently before use.
9. Incomplete washing will adversely affect the outcome and assay precision.
10. Do not use a humidified chamber for 37°C incubations, as this may affect assay performance.
11. 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.
12. 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.
13. Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
14. Do not eat, drink, smoke or apply cosmetics where immunodiagnostic materials are being handled.
15. Do not pipette by mouth.
16. Use a clean disposable pipette tip for each reagent, Standard, Control or specimen.
17. Cover working area with disposable absorbent paper.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit contains 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. MSDS sheets are available through our website or it can be sent upon request. 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.

REFERENCES

1. Martin, D.A., Muth, D.A., Brown, T., Johnson, A.J., Karabatsos,R, Roehrig, J.T. 2000. Standardization of Immunoglobulin M Capture Enzyme-Linked Immunosorbent Assays for Routine Diagnosis of Arboviral Infections. J. Clin. Microbiol. 38(5):1823-1826.
2. Davis, B.S., Chang, G-J. J, Cropp, B., Roehrig, J.T., Martin, D.A., Mitchell, C.J., Bowen, R., Bunning, M.L. 2001. West Nile Virus Recombinant DNA Vaccine Protects Mouse and Horse from Virus Challenge and Expresses In Vitro a Noninfectious Recombinant Antigen That Can Be Used In Enzyme-Linked Immunosorbent Assays. J. Virology, 75 (9): 4040-4047.
3. Johnson, A.J., Martin, D.A., Karabatsos,R, Roehrig, J.T. 2000. Detection of Anti-Arboviral Immunoglobulin G by Using a Monoclonal Antibody-based Enzyme-Linked Immunosorbent Assay. J. Clin. Microbiol. 38(5):1827-1831.
4. Han LL, Popovici F, Alexander, Jr. JP, et al. 1999. Risk factors for West Nile virus infection and meningoencephalitis, Romania, 1996. Journal of Infectious Diseases 179:230-233.
5. Komar N. West Nile viral encephalitis. 2000. Revue Scientifique et Technique 191:66-76.
6. Lanciotti RS, Roehrig JT, Deubel V, et al. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science. 286(5448):2333-2337.
7. Nash D, Mostashari F, Fina A, et al. 2001. The outbreak of West Nile virus infection in the New York City area in 1999. New England Journal of Medicine 3441:1807-1814.

ISO 13485
ISO 9001

Diagnostic Automation/ Cortez Diagnostics, Inc.
23961 Craftsman Road, Suite E/F,
Calabasas, California 91302 USA

Date Adopted	**2020-04-15**
Cat # 8400-25	**AccuDiag™- West Nile IgG ELISA -2013**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands.** www.cepartner4u.eu
Revision B Date: 2018-12-14	