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User's Manual

West Nile IgM ELISA Kit

REF **DEIA1982M**

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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 West Nile Virus IgM ELISA Kits are qualitative immunoassays for detection of human antibodies in serum or plasma to West Nile Virus.

General Description

West Nile virus belongs to the family of flaviviruses along with Dengue virus, Yellow Fever virus and TBE virus. Birds such as ravens and sparrows represent the natural reservoirs for arthropod-borne viruses. Mosquitos of the genus Culex, Aedes, and IOchlerotatus are the main vectors of West Nile virus to humans. The RNA-genome of West Nile virus is surrounded by a spherical capsid of icosahedral symmetry. The capsid is enclosed in a lipid membrane. The envelope protein E, embedded in the outer membrane, is highly immunogenic. West Nile Virus was discovered in 1937 in the West Nile district of Uganda. In large parts of Africa, eastern Europe, and Asia, infections caused by West Nile virus have long been recorded. Since the 1990s, an increased number of epidemic outbreaks have occurred in Europe, Australia, and North America. The incubation period ranges from 3-14 days. Infections with West Nile virus are usually asymptomatic. In mild cases, flu-like symptoms such as fever, headaches, myalgia, and arthralgia as well as gastrointestinal symptoms may result (West Nile fever). The virus is able to pass the blood-brain barrier, and, therefore, in rare cases a West Nile virus infection can be accompanied by encephalitis, meningitis, or acute paralysis. As outbreaks increased in Europe, Australia, and America, severe cases of disease have also increased. These severe neurological disease forms mainly occur in elderly or immunocompromised individuals and can lead to death or permanent injury.

Principles of Testing

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay which is particularly suited to the detection of antibodies. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the West Nile Virus IgM ELISA Kit microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies are present in a sample, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the antigen-antibody complexes. The colorless substrate p-nitrophenylphosphate is then converted into a colored product p-nitrophenol. The signal intensity of this reaction product is proporational to the concentration of the antibody in the sample and is measured photometrically.

Reagents And Materials Provided

Test Components	Pieces / Volume
Break apart microtiter test strips each with eight antigen coated single wells, (altogether 96) MTP, 1 frame. The coating material is inactivated.	12 pieces
Standard serum (ready-to-use) STD, Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen), and anti-HCV Ab; preservative: <0.1% sodium azide; coloring: Amaranth O	2 x 2ml
Negative control serum (ready-to-use) NEG, Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen), and anti-HCV Ab; preservative: <0.1% sodium azide; coloring: Lissamin Green V	2ml
Anti-human IgM conjugate (ready-to-use) APC, Anti-human IgM polyclonal antibody, Conjugated to alkaline phosphatase, stabilized with protein stabilization solution; preservative: <0.1% methylisothiazolone, <0.1% bromnitrodioxane	13ml
Washing solution concentrate (sufficient for 1000ml) WASH, Sodium chloride solution with Tween 20 and 30mM Tris/HCl, pH 7.4; preservative: <0.1% sodium azide	33.3ml
Dilution buffer (ready-to-use) DILB, Protein-containing phosphate buffer with Tween 20; preservative: <0.1% sodium azide; coloring: 0.01g/l bromphenol blue	2 x 50ml
Stopping solution (ready-to-use) STOP, <0.1N sodium hydroxide, 40mM EDTA	15ml
Substrate (ready-to-use) pNPP, Para-nitrophenylphosphate in solvent-free buffer; preservative: <0.1% sodium azide	13ml
Quality control certificate with standard curve and evaluation table INFO, (quantification of antibodies in IU/ml or U/ml)	2 pages

Materials Required But Not Supplied

1. Common laboratory equipment
2. Photometer for microtiter plates with filter, wavelength 405nm, recommended reference wavelength 620nm-690nm (e.g. 650nm)
3. Microtiter plate washer
4. Incubator 37°C
5. Moist chamber
6. Distilled water
7. Optional: ELISA control
8. For IgM detection: CD Rf-Absorbent (product no. Z200, 20ml)

Storage

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	Unopened After opening at 2-8°C in closed aluminum bag with desiccant	See expiry date Minimum shelf life: four weeks
Control sera/ Standard sera	Unopened / after opening at 2-8°C	See expiry date
Conjugate	Unopened / after opening at 2-8°C	See expiry date
Dilution buffer	Unopened / after opening at 2-8°C	See expiry date
Washing solution	Unopened / after opening at 2-8°C Working dilution at 2-8°C Working dilution at room temperature	See expiry date 2 weeks 1 week
Substrate	Unopened / after opening at 2-8°C	See expiry date
Stopping solution	Unopened / after opening at 2-8°C	See expiry date

Specimen Collection And Preparation

Specimen preparation and Storage

Lipemic, hemolytic, or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

Dilution of Samples

Interference by rheumatoid factors: Rheumatoid factors are autoantibodies mainly of the IgM class which preferentially bind to IgG immune complexes. The presence of non-specific rheumatoid factor IgM antibodies can lead to false-positive results in the IgM assay. Furthermore, the possibility exists that weak-binding pathogen-specific IgM antibodies may be displaced by stronger-binding IgG antibodies leading to a false-negative IgM result. Therefore, it is necessary to pretreat samples with rheumatoid factor absorbent prior to specific IgM detection (CD Rf-Absorbent, product no. Z200, 20ml/100 tests). Rf-absorption is performed by incubating sample in Rf-dilution buffer for 15 minutes at room temperature or overnight at 4°C. The test procedure is described in a separate instruction manual.

Before running the test, rheumatoid factor absorbent (V1) must be diluted 1:4 in dilution buffer (V2).

add 200ul Rf-Absorbent to 800ul Rf-dilution buffer (1:4)

Samples (V4) must be diluted in this Rf-dilution buffer (V3):

add 10ul sample to 1000ul Rf-dilution buffer (1:100)

After dilution and before pipetting into the microtiter plate, samples must be mixed thoroughly to prepare a homogenous solution.

Sample Storage

Samples should not be stored for more than 7 days at 2-8°C. Extended storage is possible at ≤ -20°C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2-8°C for one week.

Reagent Preparation

Bring all reagents to room temperature before use.

1. Microtiter Test Strips: The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate, please cut off the top of the marked side only in order to guarantee proper resealing. Take unrequired wells out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.
2. Control Sera / Standard Sera (ready-to-use): Control and standard sera are ready-to-use and must not be diluted further. For each test run – independent of the number of microtiter test strips to be used – control and standard sera must be included. Standard and cut-off sera should be set up in duplicate. Do not treat control sera with Rf-absorbent.
3. Anti-human IgM AP-Conjugate (ready-to-use): The required conjugate concentration (+, ++, +++) is indicated on the quality control certificate. Please refer also to the specification on the label.
4. Washing Solution (Concentrate): Dilute washing buffer concentrate (V1) 1:30 with distilled water to a final volume of V2.

   Example:

   33.3ml Buffer concentrate (V1) + 1000ml Final volume (V2)

   1.0ml Buffer concentrate (V1) + 30ml Final volume (V2)
5. Dilution Buffer for Samples (ready-to-use).
6. Substrate (ready-to-use) Substrate in unopened bottle may have a slight yellow color which does not reduce the quality of the product!
7. Stopping Solution (ready-to-use).

Assay Procedure

Manual Test Procedure

1. Place the required number of wells in the frame and prepare a protocol sheet.
2. Add each 100ul of diluted sample or ready-to-use controls into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

   A1 Substrate blank

   B1 Negative control

   C1 Standard serum

   D1 Standard serum

   E1 Sample 1 . . .

   F1 Sample 2 . . .
3. Sample incubation for 60 minutes (+/- 5 min.) at 37°C(+/- 1°C) in moist chamber.
4. After incubation wash all wells with washing solution (by automated washer or manually):

   - aspirate or shake out the incubation solution

   - fill each well with 300ul washing solution

   - aspirate or shake out the washing solution

- repeat the washing procedure 3 times (altogether 4 times!)

- dry by tapping the microtiter plate on a paper towel

5. Addition of conjugate Add 100ul of the ready-to-use IgM conjugate to the appropriate wells (except substrate blank).
6. Conjugate incubation for 30 minutes (+/- 1 min.) at 37°C (+/- 1°C) in moist chamber.
7. After incubation wash all wells with washing solution (see above).
8. Addition of substrate Add 100ul of ready-to-use substrate solution to each well (including well for substrate blank!).
9. Substrate incubation for 30 minutes (+/- 1 min.) at 37°C (+/- 1°C) in moist chamber.
10. Stopping of the reaction Add 100ul stopping solution to each well, shake microtiter plate gently to mix.
11. Read extinction Read optical density (OD) within 60 minutes at 405nm against substrate blank, reference wavelength between 620nm and 690nm (e.g. 650nm).

Automated Test Procedure

West Nile IgM ELISA Kit is suited for processing on automats. The automated processing is performed analogous to manual use. Please note that under special working conditions internal laboratory adaptations of the substrate incubation times may be necessary.

Quality Control

For the periodic verification of the test method, in order to fulfill the requirements of laboratory internal quality management systems, we recommend using CD ELISA controls to determine precision and accuracy of West Nile IgM ELISA test runs.

Criteria of Validity

- The substrate blank must be <0.25 OD.

- The negative control must be negative.

- By use of quantitative West Nile IgM ELISA Kit, the mean OD value (after subtraction of the substrate blank!) of the standard serum must be within the validity range which given on the lot-specific quality control certificate.

- By use of qualitative West Nile IgM ELISA Kit, the OD value of the positive control and the mean OD value of the cut-off serum must be within the validity ranges which are given on the lot-specific quality control certificate of the kit (after subtraction of the substrate blank!).

- The variation of OD values of the standard serum or cut-off serum must not be higher than 20%.

If these criteria are not met, the test is not valid and must be repeated.

Calculation

The mathematical curve fitting for antibody quantification with West Nile IgM ELISA Kit is based on the 4-parameter logistic (4PL) function.

$$Activity\ (U/ml) = e^{C-\frac{1}{B}\ln\left(\frac{D-A}{OD(Patient)*F-A}-1\right)}$$

The 4 parameters A, B, C, and D are representative for the exact shape of the standard curve:

Parameter A: Lower asymptote (OD)

Parameter B: Slope of the curve

Parameter C: Inflection point

Parameter D: Upper asymptote (OD)

For the adaptation of the test level to the given 4PL standard curve, the correction factor F is calculated by dividing the standard reference OD value indicated on the quality control certificate with the measured, and consequently test run-specific, standard OD value.

$$F = \frac{STD\ reference\ OD\ value}{measured\ STD\ OD\ value}$$

By multiplying the OD values obtained from samples with the correction factor F, the level of each individual test run is adjusted to the given 4PL standard curve. Thereby, interassay deviations are compensated for and antibody activities can be directly evaluated from the 4PL standard curve.

After subtraction of the substrate blank from all measured OD values and calculation of the mean OD value of the standard serum (STD), tested in duplicate, a range of possibilities are available for the evaluation of antibody activities from the optical measurement signals (OD) of samples.

Interpretation Of Results

Borderline Ranges

The borderline ranges of the West Nile IgM ELISA Kit are specified on the quality control certificates and indicate the range of borderline test results. Values below this range indicate a negative value; values above the borderline range indicate a positive value.

Limits of Quantification

The limits of quantification are specified on the quality control certificate of the West Nile Virus IgM ELISA Kit. The linearity of dilution within this range has been demonstrated in comprehensive evaluation studies. In case a sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.

Precautions

1. Optimum results can only be achieved if the instructions are strictly followed. The components must not be exchanged for reagents of other manufacturers. Standard and control sera of West Nile IgM ELISA Kit are defined exclusively for the test kit to be used and must not be used in other lots.
2. There are three different conjugate concentrations for each immunoglobulin class (IgM), indicated on the label as + (low), ++ (medium), and +++ (high). Dilution or alteration of the reagents may result in a loss of

sensitivity. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination.

3. Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the tubes containing control sera before use and also all samples after dilution (e.g. by using a vortex mixer).
4. Be sure to pipette carefully and comply with the given incubation times and temperatures. Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate, or substrate can result in different preincubation times which may influence the precision and reproducibility of the results. Avoid exposure of reagents to strong light during storage and incubation.
5. Adequate washing avoids non-specific reagent binding. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure, ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!
6. Reagents must be tightly closed after use to avoid evaporation and contamination. Take care not to mix up the caps of the bottles and/or vials.
7. The West Nile IgM ELISA Kit is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.
8. The West Nile IgM ELISA Kit is designed for use by qualified personnel who are familiar with good laboratory practice. All kit reagents and samples should be handled carefully using established good laboratory practice.
9. This kit contains human blood components. Although all control and cut-off sera have been tested and found negative for anti-HIV ab, HBs-Ag (Hepatitis B-Virus surface Antigen), and anti-HCV ab, they should be considered potentially infectious.
10. Do not pipette by mouth.
11. Do not smoke, eat, or drink in areas in which samples or kit reagents are handled.
12. Wear disposable gloves, laboratory coat, and safety glasses while handling kit reagents or samples. Wash hand thoroughly afterwards.
13. Samples and other potentially infectious material should be decontaminated after the test run.
14. Reagents should be stored safely and be inaccessible to unauthorized access, e.g. children.