General information

This diagnostic kit is designed to detect antibodies directed against the envelope protein (pr-E) of Flaviviruses by competitive ELISA.

Initially designed for the detection of anti-West-Nile Virus (WNV) antibodies, this test has been proven to detect a broad spectrum of Flaviviruses such as the WNV but also the USUV, JEV, TBEV, ZIKAV and DENV (literature references available upon request).

This test can be used with serum or plasma samples from equids, birds, humans (for research use only) or from any other susceptible species.

Description and principle

Microwells are coated with a purified extract of the West Nile virus.

Samples to be tested and controls are added to the wells. The anti-pr-E antibodies, if present, form an antigen-antibody complex.

An anti-pr-E antibody peroxidase (HRP) conjugate is added to the wells. It fixes to the remaining free pr-E epitopes, forming an antigen-conjugate-HRP complex.

After washing in order to eliminate the excess conjugate, the Substrate solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested:

- in the absence of antibodies, a blue coloration appears which becomes yellow after addition of the Stop solution.
- in the presence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Kit components

Reagents*
Microplates coated with purified extract.
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 2
Concentrated Wash Solution (20X)
Substrate Solution
Stop Solution (0,5 M)

** Quantities supplied are indicated on the kit label.*

1. The Conjugate, the Controls and the Substrate solution must be stored at 5°C (± 3°C).
2. The other reagents can be stored between +2°C and +26°C.
3. For detailed storage conditions of opened and/or diluted components, please refer to www.innovative-diagnostics.com/storage-conditions/
4. Wash and Stop solutions can be used for the entire IDvet product range. Substrate solutions and Dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

1. Mono or multi-channel micropipettes capable of delivering volumes of 10 µL, 100 µL, and 500 µL.
2. Disposable tips.
3. Distilled or deionized water.
4. 96-well microplate
5. Manual or automatic wash system.
6. 96-well microplate reader.

Precautions

1. Do not pipette by mouth.
2. Contains components that can be harmful to the skin and eyes and may cause sensitisation by skin contact. Avoid contact with skin and eyes. Use protective lab coat, one-way gloves and safety glasses. The Stop solution (0.5 M acid) may be harmful if swallowed.
3. Do not expose the Substrate solution to bright light or to oxidizing agents.
4. All waste should be properly decontaminated prior to disposal. Dispose in accordance with local regulations.

Please refer to the Material Safety Data Sheet, available upon request at info@innovative-diagnostics.com, for more detailed information.

Sample preparation

In order to avoid differences in incubation times between specimens, it is possible to prepare a 96-well microplate containing the test and control specimens, before transferring them into an ELISA microplate using a multichannel pipette.

Wash Solution preparation

If necessary, bring the **Wash Concentrate (20X)** to room temperature and mix thoroughly to ensure that the Wash Concentrate (20X) is completely solubilized.

Prepare the **Wash Solution (1X)** by diluting the **Wash Concentrate (20X)** to 1:20 in distilled/deionized water.

The quality of the wash step may influence results. Ensure that wells are completely empty between washes. If using an automatic washer, it is extremely important to correctly parameter the machine (mode, type of aspiration, aspiration height). For more information, please consult the "IDvet Washing Guide", available upon request.

Testing procedure

Allow all the reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or vortexing.

1. In the ELISA microplate, add:
   - 50 µL of **Dilution Buffer 2** to each microwell,
   - 50 µL of the **Positive Control** to wells A1 and B1,
   - 50 µL of the **Negative Control** to wells C1 and D1,
   - 50 µL of **each sample to be tested** in the remaining wells.
2. Cover the plate and incubate **90 min** ± **6 min** at 21°C (± 5°C).
3. Empty the wells. Wash each well 3 times with at least 300 µL of the **Wash Solution.** Avoid drying of the wells between washes.
4. Prepare the **Conjugate 1X** by diluting the **Concentrated Conjugate 10X** to 1:10 in **Dilution Buffer 2.**
5. Add 100 µL of the **Conjugate 1X** to each well.
6. Cover the plate and incubate **30 min** ± **3 min** at 21°C (± 5°C).
7. Empty the wells. Wash each well 3 times with at least 300 µL of the **Wash Solution.** Avoid drying of the wells between washes.
8. Add 100 µL of the **Substrate Solution** to each well.
9. Cover the plate and incubate **15 min** ± **2 min** at 21°C (± 5°C) in the dark.
10. Add 100 µL of the **Stop Solution** to each well in the same order as in step No. 8, to stop the reaction.
11. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean value of the Negative Control O.D. (OD_{NC}) is greater than 0.700.

$$\mathbf{OD_{NC} > 0.700}$$

✓ the mean value of the Positive Control (OD_{PC}) is less than 30 % of the OD_{NC}.

$$\mathbf{OD_{PC}/OD_{NC} < 0.3}$$

Interpretation

For each sample, calculate the S/N percentage (S/N%):

$$\mathbf{S/N\ \% = \frac{OD_{sample}}{OD_{NC}}\ x\ 100}$$

Samples presenting a S/N%:

- less than or equal to 40% are considered positive.
- less than or equal to 50% and greater than 40% are considered doubtful.
- greater than 50% are considered negative.

Result	Status
S/N % ≤ 40%	POSITIVE
40% < S/N % ≤ 50%	DOUBTFUL
S/N % > 50%	NEGATIVE

Note: The IDSoft™ data analysis program is available free-of-charge. Please contact support.software@innovative-diagnostics.com for more information.

This software program can calculate many parameters (validation criteria, S/P or S/N values, titers, vaccination age, groups) and offers a graphic representation of the serological profiles of the animals tested).

Certified management system

ID Screen®
Flavivirus Competition

Competitive ELISA for the detection of antibodies against the Flavivirus prE protein in serum or plasma from equids, birds, humans (for research use only) or any other susceptible species

For *in vitro* use

December 2023:

- **New commercial denomination:** 'ID Screen® Flavivirus Competition' instead of 'ID Screen® West Nile Multi-species' **to reflect the tool's diagnostic performance and broad detection spectrum, useful for screening. The kit remains unchanged (with the same product code), and with no change in the testing procedure, validation and interpretation criteria.**
- Human samples testing now available for Research Use Only (RUO), without modifications in the instructions for use (protocol and cut-off values). Please refer to publications and peer-reviewed references for more information.

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