General Information

This diagnostic kit is designed to detect horse and avian antibodies directed against the West Nile Virus envelope protein (pr-E) by competitive ELISA.

The monoclonal antibody used in the kit cross-reacts with other Japanese Encephalitis viruses and the Tick-borne encephalitis virus.

Please contact IDvet for use in other 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 solution 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 antigen.
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. Components bearing the same name (*wash solution, dilution buffers*) can be used for the entire IDvet product range.

Materials required but not provided

1. Mono or multi-channel micropipettors capable of delivering volumes of 10 µl, 100 µl, and 200 µl.
2. Disposable tips.
3. 96-well microplate reader.
4. Distilled or deionized water.
5. Manual or automatic wash system.

Precautions

1. Do not pipette by mouth.
2. The substrate solution can be irritating to the skin.
3. The stop solution (0,5 M) may be harmful if swallowed. It may cause sensitisation by skin contact (**R22-43**). Avoid contact with skin (**S24-37**).
4. Do not expose the substrate solution to bright light nor to oxidating agents.
5. All single-use material used for the assays should be decontaminated by immersion in freshly prepared 5% sodium hypochlorite for minimum 1 hour before elimination, or by autoclaving at 120°C.

Sample Preparation

In order to avoid differences in incubation times between specimens, it is possible to prepare a 96-well plate 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.

Testing Procedure

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

1. 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. Incubate **90 min** ± **6 min** at 21°C (± 5°C).
3. Empty the wells. Wash each well 3 times with approximately 300 µl of the **Wash Solution.** Avoid drying of the wells between washings.
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. Incubate **30 min** ± **3 min** at 21°C (± 5°C).
7. Empty the wells. Wash each well 3 times with approximately 300 µl of the **Wash Solution.** Avoid drying of the wells between washings.
8. Add 100 µl of the **Substrate Solution** to each well.
9. 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 order 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.

OD_{NC} > 0.700

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

OD_{PC} / OD_{NC} < 0,3

Interpretation

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

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

Samples presenting a S/N%:

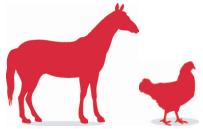
- less than or equal to 40% are considered positive.
- less than or equal to 50% and greater than 40% are considered doubtful.
- greater than 50% are considered negative.

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

IDvet
Innovative Diagnostics

Certified management system

ID Screen®
West Nile Competition Multi-species



Kit for the detection of West Nile Virus anti-pr-E antibodies in horse and avian sera by competitive ELISA.

For *in vitro* use

WNC ver 1014 GB

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