General Information

The West Nile Virus (WNV), which causes encephalitis in infected humans and horses, is maintained in nature by a mosquito vector and a bird reservoir host.

This test allows for the detection of anti-prE IgM antibodies in horse serum or plasma.

This kit is an Ig**M** **A**ntibody **C**apture ELISA (MAC). It is specific for the detection of IgM antibodies and therefore allows for detection of recent infections.

Description and Principle

Wells are coated with anti-horse IgM polyclonal antibody.

Samples to be tested and controls are added to the microwells. IgM antibodies are captured on the plate.

Plates are washed and a West Nile Virus antigen is added to the microwells. It fixes to the anti-WNV IgM captured on the plate.

After washing in order to eliminate the excess antigen, a monoclonal antibody anti-WN prE-conjugated to HRP is added. It fixes to the antigen previously captured on anti-WN IgM antibodies. 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 presence of antibodies, a blue coloration appears which becomes yellow after addition of the stop solution.
- in the absence of antibodies, no coloration appears.

The microplate is read at 450nm.

Kit Components

Reagents*
Microplates coated with anti-horse IgM antibody
WNV antigen (ready-to-use)
Anti-WNV prE HRP conjugate (10X)
Positive Control
Negative Control
Serum Dilution Buffer 18
Conjugate Dilution Buffer 14
Wash Concentrate (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. 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 https://www.id-vet.com/fr/support/faq
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 pre-dilution microplate.
5. Manual or automatic wash system.
6. 96-well microplate reader.

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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 nor to oxidizing agents.
4. All wastes 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.

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature (21°C ± 5°C) and mix thoroughly to ensure that the Wash Concentrate 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 reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or vortexing.

1. In a pre-dilution plate, add:
   - 25 µl of the **Negative Control** to wells A1, B1,
   - 25 µl of the **Positive Control** to wells C1, D1,
   - 25 µl of **each sample to be tested** in the remaining wells,
   - 225 µl of **Dilution Buffer 18** to all wells.
2. In the ELISA microplate, transfer:
   - 100 µl of the **pre-diluted Negative Control** to wells A1, B1 and A2, B2.
   - 100 µl of the **pre-diluted Positive Control** to wells C1, D1 and C2, D2.
   - 100 µl of each **pre-diluted sample**. **Each sample must be deposited twice in adjacent even and odd-numbered wells – please refer to Figure 1 below).**
3. Cover the plate and incubate **60 min** ± **6 min** at 37°C (± 3°C).
4. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.
5. Add 50 µl of the **ready to use Ag West Nile** to the **even-numbered columns**. Add 50µl of **Dilution Buffer 18** to the odd-numbered columns.
6. Cover the plate and incubate **overnight** (16-20h) at 21°C (± 5°C).

	D18	WN-Ag	D18	WN-Ag	D18	WN-Ag	D18	WN-Ag	D18	WN-Ag	D18	WN-Ag
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	NC	NC	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
B	NC	NC	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
C	PC	PC	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	PC	PC	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
E	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
H	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44

Figure 1: Plate map. Samples are deposited in duplicate in adjacent even and odd-numbered wells. NC=negative control. PC=positive control. S=sample. D18=Dilution Buffer 18. WN-Ag= West Nile antigen.

7. Prepare the **Conjugate 1X** by diluting the **Conjugate 10X** to 1:10 in the **Dilution Buffer 14.**
8. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.
9. Add 50 µl of the **Conjugate 1X** to each well.
10. Cover the plate and incubate **30 min** ± **3 min** at 21°C (± 5°C).
11. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.
12. Add 100 µl of the **Substrate Solution** to each well.
13. Cover the plate and incubate **15 min** ± **2 min** at 21°C (± 5°C) in the dark.
14. Add 100 µl of the **Stop Solution** to each well, in the same order as in step No. 12, to stop the reaction.
15. Read and record the OD at 450 nm.

Validation

Calculate the net OD results (to be used for the validation and interpretation steps):

$$\mathbf{net\ OD} = \mathbf{OD_{even\ well}} - \mathbf{OD_{odd\ well}}$$

The test is validated if:

✓ the mean value of the net Positive Control OD (net OD_{PC}) is greater than 0.350.

$$\mathbf{net\ OD_{PC}} > \mathbf{0.350}$$

✓ the ratio of the mean values of the net Positive and Negative Control ODs (net OD_{PC} and net OD_{NC}) is greater than 3. In this formula, use the absolute value of the net OD_{NC}.

$$\mathbf{net\ OD_{PC}} / |\mathbf{net\ OD_{NC}}| > 3$$

Interpretation

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

$$\mathbf{S/P\ \%} = \frac{(\mathbf{net\ OD_{sample}} - \mathbf{net\ OD_{NC}})}{(\mathbf{net\ OD_{PC}} - \mathbf{net\ OD_{NC}})} \mathbf{x\ 100}$$

Samples presenting an S/P %:

- Less than or equal to 35% are considered negative.
- Between 35% and 45% are considered as doubtful.
- Greater than or equal to 45% are considered positive.

Result	Status
S/P % ≤ 35%	NEGATIVE
35% < S/P% < 45%	DOUBTFUL
S/P % ≥ 45%	POSITIVE

Note 1: if the sample control well ($OD_{odd\ well}$) is greater than the mean value of the net positive control OD (net OD_{PC}), the result cannot be interpreted.

Note 2: The IDSoft™ data analysis program is available free-of-charge. For more information, please contact support.software@innovative-diagnostics.com

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®
West Nile IgM Capture

Capture ELISA (biwell format) for the detection of IgM antibodies directed against the West Nile Virus in horse serum or plasma

For *in vitro* use

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