VetMAX™ West Nile Virus Kit

TaqMan® real-time RT-PCR for detection of the West Nile virus

Catalog Number WNPEX050

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Technology	Species	Nucleic acid isolated from matrices	Test type
Real-time RT-PCR (RNA) – Duplex – Endogenous/exogenous IPC	Horse	Whole blood, serum, plasma Cell culture supernatant Feces Tracheal and cloacal swabs Organs (cervix...) Cerebrospinal fluid	Individual

⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

⚠ **WARNING! POTENTIAL BIOHAZARD.** Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Information about the product

Description of the product

The **Applied Biosystems™ VetMAX™ West Nile Virus Kit** is a molecular diagnostic tool for detecting West Nile virus by real-time RT-PCR.

Each RNA sample obtained after extraction is analyzed in a single well; the same well is used to specifically detect the viral RNA of West Nile virus and an IPC (Internal Positive Control). The kit simultaneously detects an endogenous IPC in cellular samples and an exogenous IPC to be added to non-cellular samples. A positive IPC reflects both the efficiency of extraction and the absence of inhibitor in the samples.

It can be used on viral RNA extracted from **whole blood, serum and plasma, cell culture supernatant, feces, tracheal and cloacal swabs, organs,** and **cerebrospinal fluid**.

Complete protocols for viral RNA extraction from these matrices are available upon request from Technical Support.

Kit contents and storage

The **VetMAX™ West Nile Virus Kit** contains components that can be used for detecting in duplex the West Nile virus and an IPC. Upon receipt, the whole kit must be stored at **–30°C to –10°C**. After initial use of a component, store it according to the following recommendations:

Component	Description	Volume (50 reactions)	Storage	
			Upon receipt	After initial use
3 - Mix West Nile (Green tube)	Mix for TaqMan® RT-PCR. Contains: • The detection system for the West Nile virus target, including a TaqMan® probe labeled **FAM™ – NFQ** (Non-Fluorescent Quencher). • The detection system for IPCs, including several TaqMan® probes labeled **VIC™ – TAMRA™**. • Buffer, reverse transcriptase and real-time PCR enzyme.	2 × 500 µL	–30°C to –10°C	–30°C to –10°C
4a - EPC West Nile (Brown tube)	**External Positive Control:** Positive control for West Nile virus It consists of **already extracted** nucleic acid to be amplified during real-time RT-PCR.	90 µL	–30°C to –10°C	–30°C to –10°C
5 - IPC West Nile (Yellow tube)	**Internal Positive Control:** Exogenous internal control **to be added to each non-cellular and feces sample and each control** in the lysis step of the extraction.	250 µL	–30°C to –10°C	–30°C to –10°C

NOTE: For small extraction series, it is recommended that the IPC West Nile be aliquoted to avoid more than 3 cycles of freezing/thawing (a minimum volume of 50 µL).

Extraction and amplification controls

The **VetMAX™ West Nile Virus Kit** contains two controls, enabling validation of the extraction and the amplification of the viral RNA:

4a - EPC West Nile: positive control for West Nile

A positive control, **already extracted,** for amplification during the real-time RT-PCR.

A positive result within the specified C_t range validates the amplification of the West Nile target by real-time RT-PCR.

5 - IPC West Nile: extraction internal control (optional use depending on the type of sample)

Positive control **to be added to each non-cellular and feces sample during the lysis step** of the nucleic acid extraction. For cellular samples, nucleic acid extraction validation is performed using an endogenous IPC present in each cellular sample.

A positive IPC result with a compliant value in a cellular sample (for an endogenous IPC) or within the specified C_t range in a sample validates the extraction of this non-cellular or feces sample (for an exogenous IPC), whether positive or negative for the target pathogen, thus eliminating false negatives and verifying the effect of the inhibitors.

We recommend including two negative controls to confirm correct analysis:

NCS: negative extraction control

This control consists of components used in the extraction without addition of the sample (sample volume can be replaced by the buffer used in the sample preparation or by DNase/RNase-free water) that undergoes the same treatment as the samples: nucleic acid extraction (with or without IPC added) and real-time RT-PCR.

A negative result for the West Nile Virus and the endogenous IPC (for cellular samples) confirms the absence of contamination during the extraction and the real-time RT-PCR.

NC: negative amplification control

This control consists of an amplification mix added to the plate during real-time RT-PCR preparation, as well as 5 µL of DNase/RNase-free water to adjust the reaction to 25 µL.

A negative result for the West Nile virus and the IPC confirms the absence of contamination during real-time RT-PCR reaction preparation.

Materials required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**.

- Precision micropipettes (range of 1 µL to 1000 µL) with DNase/RNase-free filtered tips
- DNase/RNase-free water
- 1X TE buffer
- 1X PBS buffer
- A real-time PCR thermal cycler capable of detecting the following fluorophores:
  - FAM™ (emission maximum: λ515 nm)
  - VIC™ (emission maximum: λ554 nm)
- Optical-quality consumables compatible with the thermal cycler used: PCR 96-well plates, PCR strips (8 or 12 wells), microtubes or capillaries; suitable plate covers or caps for capping

Analysis procedure

The real-time PCR reaction volume is 25 µL:

- **3 - Mix West Nile**: 20 µL per analysis
- **Extracted RNA:** 5 µL per analysis

Extraction of viral RNA

RNA must be extracted from the samples for real-time RT-PCR analysis.

For non-cellular and feces samples, add **5 µL** of **5 - IPC West Nile** to each sample to be extracted and the NCS in the lysis step of the nucleic acid extraction.

NOTE: For information about extraction methods that are compatible with and validated for the VetMAX™ West Nile Virus Kit, please contact Technical Support.

Preparation of the real-time RT-PCR

1. Create an analysis plan for distribution of the mixes and samples. Keep the positive control (EPC) away from the other samples if possible.
2. Thaw the tube of **3 - Mix West Nile** at **2°C to 8°C on ice** or on a refrigerated rack.
3. Mix the tube of **3 - Mix West Nile** by shaking gently, then centrifuge briefly.
4. Add **20 µL of 3 - Mix West Nile** to each PCR plate well, PCR strip or capillary used.
5. Add RNA from the samples and controls to the reaction mix, according to the pre-defined analysis plan:

Type of analysis	Component	Sample volume
Sample for analysis	RNA extracted from sample	5 µL
Positive amplification control	**4a - EPC West Nile**	5 µL
Negative lysis control (NCS)	Extracted NCS	5 µL
Negative amplification control (NC)	DNase/RNase-free water	5 µL

6. Cover the PCR plate, PCR strips or capillaries with an adhesive plate cover or suitable caps.

Amplification by real time RT-PCR

1. Create the following detectors on the thermal cycler:

	Reporter	Quencher
WN	FAM™	NFQ (Non-Fluorescent Quencher)
IPC WN	VIC™	TAMRA™[1]
Passive reference: ROX™[1]		

[1] The fluorophores TAMRA™ and ROX™ are required for real-time RT-PCR analysis if the thermal cycler is capable of detecting them. For other thermal cyclers, the absence of detection of these fluorophores does not affect the real-time RT-PCR analysis.

2. Assign the **WN** detector and the **IPC WN** detector to each sample well used in the analysis.
3. Set up the following real-time RT-PCR program for the analysis:

	Step repetitions	Temperature	Duration
Step 1	×1	45°C	10 minutes
Step 2	×1	95°C	10 minutes
Step 3	×40	95°C	15 seconds
		65°C[1]	45 seconds

[1] Collection of fluorescence data during the 65°C – 45 seconds stage.

4. Place the PCR plate, the PCR strips or the capillaries in the thermal cycler and run the real-time RT-PCR.

Analysis of the results

Analysis of the raw data

Refer to the recommendations of the thermal cycler manufacturer for the analysis of the raw data.

1. Position the threshold limits separately for each target of the real-time RT-PCR.
2. For each detector, interpret the results according to the sample C_t values obtained as recommended below.

Validation

The test is validated if the following criteria are met:

	West Nile detector	West Nile IPC detector	Validation
EPC West Nile	$C_t = C_{t\,QC}$ West Nile of **4a - EPC West Nile** ± $3C_t$[1]	For the endogenous IPC: $C_t < 40$ or $C_t > 40$[2]	PCR validated
NCS	$C_t > 40$	If endogenous IPC (without exogenous IPC added): $C_t > 40$	Extraction validated
		If exogenous IPC added: $C_t = C_{t\,QC}$ IPC of **5 - IPC West Nile** ± $3C_t$[3]	
NC	$C_t > 40$	$C_t > 40$	PCR components validated

[1] Refer to the values listed in section 2.1 "EPC" of the Certificate of Analysis of the lot used for the test.
[2] The IPC value in the EPC should not be used for test validation.
[3] Refer to the values listed in section 2.2 "IPC" of the Certificate of Analysis of the lot used for the test.

Interpretation of results

For each sample analyzed, the results should be interpreted as shown below:

For non-cellular and feces samples:

West Nile detector	West Nile IPC detector (exogenous IPC)	Interpretation
$C_t < 40$	$C_t < 40$ or $C_t > 40$	West Nile virus detected
$C_t > 40$	$C_t \leqslant C_t$ IPC of NCS + $3C_t$[1]	West Nile virus not detected
$C_t > 40$	$C_t > C_t$ IPC of NCS + $3C_t$[1]	Not validated[2]

[1] Refer to the IPC C_t value obtained for the NCS done during the same extraction series as the samples to be analyzed. The IPC C_t value obtained for this NCS must first be validated as described above.
[2] The sample will be returned as not validated due to the negative IPC.

For cellular samples:

West Nile detector	West Nile IPC detector (endogenous IPC)	Interpretation
$C_t < 40$	$C_t < 40$ or $C_t > 40$	West Nile virus detected
$C_t > 40$	$C_t < 40$	West Nile virus not detected
$C_t > 40$	$C_t > 40$	Not validated[1]

[1] The sample will be returned as not validated due to the negative IPC.

Procedure for handling non-validated samples

1. Dilute the RNA at a 1:10 dilution in 1X TE buffer.
2. Denature the diluted RNA.
3. Perform a new RT-PCR analysis on 5 µL of this dilution (after denaturation).
4. If the diluted RNA is positive for West Nile with an acceptable IPC result, the result obtained is then validated.
5. If the diluted RNA is negative for West Nile with a non-compliant IPC result, the obtained result is still not validated. In this case, repeat the nucleic acid extraction using the sample pre-diluted 1:10 in 1X PBS buffer before extraction.
6. If the result is still not validated, repeat the analysis on a new sample.

Documentation and support

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)
  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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The information in this guide is subject to change without notice.

Revision history of Pub. No. MAN0008915 (English)

Revision	Date	Description
B.0	28 June 2017	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	31 March 2014	Baseline for revision history