

Instructions for Use

RealStar[®] WNV RT-PCR Kit 2.0

03/2020 EN

RealStar[®]

WNV RT-PCR Kit 2.0

For use with

Mx 3005P™ QPCR System (Stratagene)
VERSANT® kPCR Molecular System AD (Siemens Healthcare)
ABI Prism® 7500 SDS (Applied Biosystems)
ABI Prism® 7500 Fast SDS (Applied Biosystems)
LightCycler® 480 Instrument II (Roche)
Rotor-Gene® 6000 (Corbett Research)
Rotor-Gene® Q5/6 plex Platform (QIAGEN)
CFX96™ Real-Time PCR Detection System (Bio-Rad)
CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)



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1. Intended Use

The RealStar® WNV RT-PCR Kit 2.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of West Nile virus (WNV) specific RNA.

2. Kit Components

Lid Color	Component	Number of Vials	Volume [μ l/Vial]
Blue	Master A	8	60
Purple	Master B	8	180
Green	Internal Control	1	1000
Red	Positive Control	1	250
White	Water (PCR grade)	1	500

CAUTION



Before first use check the product and its components for completeness with respect to number, type and filling. Do not use a defective or incomplete product, performance could be compromised.

3. Storage

- The RealStar® WNV RT-PCR Kit 2.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact Altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

CAUTION



Improper storage conditions may lead to a compromised product performance.

CAUTION



Do not exceed thaw-freeze-sequence and handling durations as specified in these Instructions for Use.

CAUTION



Do not use product components beyond the expiration date printed on the component label.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 8.1 Sample Preparation)
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE



Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations.

NOTE



It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).

5. Background Information

West Nile virus (WNV) is a virus species which belongs to the family *Flaviviridae*. The RNA genome of flaviviruses is single stranded and has got a positive orientation [(+) ssRNA]. The genes are all located on a single segment of around 9 – 12 kb length. The 5'-end of the genomes contain specific sequences which fold into relatively stable conformations. These so called ribosomal entry sequences allow for direct translation and protein synthesis by host ribosomes.

West Nile virus is transmitted by arthropod vectors (different mosquitoes of the genera *Culex*, *Aedes* or *Ochlerotatus*). It can infect a variety of different host species like horses, birds and finally humans. The virus was first identified in 1937 in Uganda and since then, it was continuously found in birds, horses and human in Africa, the Middle-East and Southern-Europe. 1999 the virus reached North- America. A mosquito carrying the virus was probably brought from Israel to New York. Several outbreaks in the USA have been recorded since.

In most of the cases (70-80%), the infection is asymptomatic in humans. In symptomatic cases fever with other signs and symptoms like headache, joint pains, vomiting and other rather unspecific illnesses can be observed. In few cases, around 1% of all infections, severe neurological complications will occur. These patients develop severe headaches, stiff neck, coma or paralysis as a consequence. 10% of these patients will die due to the infection with WNV.

There is no specific treatment available to cure WNV infections. Medication to reduce fever and pain can be administered and severe cases will receive supportive treatment and be hospitalised.

Virus can be detected from day 2–3 until 14–19 days post-infection in serum/plasma of patients. Interestingly, detection of the virus RNA from urine is possible even after it disappeared from the blood.

Serology is difficult as antibodies cross-react with other flaviviruses. IgM and IgG can be detected in patients 4-8 days after detection of virus in blood. The most specific serological test is the plaque reduction neutralisation test but this requires

virus culture and can only be performed by expert laboratories.

Virus RNA detection is mainly done for two different purposes. Firstly, RT-PCR is performed on samples of suspected cases to do *in-vitro* diagnostics. Here, virus load is usually high and the limit of detection of the assay is of minor concern. Secondly, in areas where WNV is endemic, transmission of the virus has occurred through transfusion of blood. Therefore, safety screening of blood donations should be done and for this very high sensitivity is needed.

NOTE



Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results.

6. Product Description

The RealStar® WNV RT-PCR Kit 2.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of West Nile virus (WNV) specific RNA.

The assay includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labeled with fluorescent reporter and quencher dyes.

Probes specific for WNV are labeled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labeled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of WNV specific RNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of three processes in a single tube assay:

- Reverse transcription of target and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labeled probes

The RealStar® WNV RT-PCR Kit 2.0 consists of:

- Master A
- Master B
- Internal Control
- Positive Control
- Water (PCR grade)

Master A and Master B contain all components (PCR buffer, reverse transcriptase, DNA polymerase, magnesium salt, primers and probes) to allow reverse transcription, PCR mediated amplification and detection of WNV specific RNA and Internal Control in one reaction setup.

6.1 Real-Time PCR Instruments

The RealStar® WNV RT-PCR Kit 2.0 was developed and validated to be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

6.2 Sample Types

The RealStar® WNV RT-PCR Kit 2.0 has been validated for use with the following sample type:

- Human EDTA Plasma

The RealStar® WNV RT-PCR Kit 2.0 has been validated using the AltoStar® Purification Kit 1.5 on the AltoStar® Automation System AM16 for nucleic acid extraction and purification.

7. Warnings and Precautions

- Before first use check the product and its components for completeness with respect to number, type and filling. Do not use a defective or incomplete product, performance could be compromised.
- Do not use other sample types! The use of other sample types may compromise the product performance.
- The presence of PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- In case the sample contains other pathogens than WNV competition with the target amplification or cross-reactivities may occur.
- Improper storage conditions may lead to a compromised product performance.
- A lack of centrifugation of the product components after thawing could lead to contamination of the components with reagent residues in the lids and as consequence to a compromised product performance.
- Do not exceed thaw-freeze-sequence and handling durations as specified in these Instructions for Use.
- Do not use product components beyond the expiration date printed on the component label.
- Improper handling of product components and samples may lead to contamination causing incorrect IVD examination results.
 - Do not interchange vial or bottle caps, as cross-contamination may occur.
 - To minimize the risk of carryover contamination store positive and/or potentially positive material separated from the kit components.
 - Use separated working areas for sample preparation/reaction setup and amplification/detection activities.
 - Always wear disposable gloves.
 - Do not open the PCR plates or tubes post amplification to avoid contamination with amplicons.
- Storage of eluates under wrong conditions may lead to degradation of the WNV target sequence.

- Do not exceed the PCR Mix storage time. This could lead to a compromised product performance.
- Always treat samples as infectious and (bio-)hazardous in accordance with safe laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.
- Dispose of hazardous and biological waste only in compliance with local and national regulations to avoid environmental contamination.
- As with any diagnostic test, results should be interpreted in consideration of all clinical and laboratory findings.
- Potential mutations within the target regions of the WNV genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogen.
- If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.
- The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.

8. Procedure

CAUTION



Improper handling of product components and samples may lead to contamination causing incorrect IVD examination results.

- Do not interchange vial or bottle caps, as cross-contamination may occur.

- To minimize the risk of carryover contamination store positive and/or potentially positive material separated from the kit components.

- Use separated working areas for sample preparation/reaction setup and amplification/detection activities.

- Always wear disposable gloves.

- Do not open the PCR plates or tubes post amplification to avoid contamination with amplicons.

8.1 Sample Preparation

Extracted RNA is the starting material for the RealStar® WNV RT-PCR Kit 2.0.

The RealStar® WNV RT-PCR Kit 2.0 was validated with human EDTA plasma using the AltoStar® Automation System AM16 in combination with the AltoStar® Purification Kit 1.5.

Alternative nucleic acid extraction systems and kits (see below) might also be appropriate. The suitability of the nucleic acid extraction procedure for use with RealStar® WNV RT-PCR Kit 2.0 has to be validated by the user.

- QIAamp® Viral RNA Mini Kit (QIAGEN)
- QIAasymphony® (QIAGEN)
- NucliSENS® easyMag® (bioMérieux)
- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

After completion of the extraction procedure the eluates in the unsealed eluate plate are stable at room temperature (max. 30 °C) for a total of 6 hours. The eluates in a sealed eluate plate can be stored at 2 °C - 8 °C for up to 24 hours before the start of a PCR reaction setup.

CAUTION



Do not use other sample types! The use of other sample types may compromise the product performance.

CAUTION



If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

CAUTION



The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

CAUTION



Always treat samples as infectious and (bio-)hazardous in accordance with safe laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.

CAUTION



The presence of PCR inhibitors (e.g. heparin) may cause false negative or invalid results.

CAUTION



This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.

CAUTION



Dispose of hazardous and biological waste only in compliance with local and national regulations to avoid environmental contamination.

CAUTION



Storage of eluates under wrong conditions may lead to degradation of the WNV target sequence.

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® WNV RT-PCR Kit 2.0 contains a heterologous Internal Control (IC), which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

- ▶ If the IC is used as a RT-PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	21 µl	252 µl

- ▶ If the IC is used as a control for the sample preparation procedure and as a RT-PCR inhibition control, add the IC during the nucleic acid extraction procedure.
- ▶ No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10 % of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.

- If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	20 µl	240 µl

CAUTION

A lack of centrifugation of the product components after thawing could lead to contamination of the components with reagent residues in the lids and as consequence to a compromised product performance.

NOTE

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.

NOTE

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

8.3 Reaction Setup

- ▶ Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ▶ Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	30 µl

- ▶ Make sure that at least one Positive and one Negative Control is used per run.
- ▶ Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.
- ▶ Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ▶ Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

After completion of the PCR Reaction Setup the PCR Mix is stable at room temperature (max. 30 °C) for 30 minutes.

CAUTION



Do not exceed the PCR Mix storage time. This could lead to a compromised product performance.

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® WNV RT-PCR Kit 2.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

- ▶ Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

9.2 Fluorescence Detectors (Dyes)

- ▶ Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
WNV specific RNA	WNV	FAM™	(None)
Internal Control (IC)	IC	JOE™	(None)

9.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Reverse Transcription	Hold	1	-	55	20:00
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			yes	55	00:45
			-	72	00:15

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® WNV RT-PCR Kit 2.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run

A diagnostic test run is **valid**, if the following control conditions are met:

Control ID	Detection Channel	
	FAM™	JOE™
Positive Control	+	+/-*
Negative Control	-	+

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run

A diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

CAUTION



As with any diagnostic test, results should be interpreted in consideration of all clinical and laboratory findings.

10.2.1 Qualitative Analysis

Detection Channel		Result Interpretation
FAM™	JOE™	
+	+*	WNV specific RNA detected.
-	+	No WNV specific RNA detected. Sample does not contain detectable amounts of WNV specific RNA.
-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high WNV RNA load in the sample can lead to a reduced or absent Internal Control signal.

11. Performance Evaluation

The analytical performance evaluation of the RealStar® WNV RT-PCR Kit 2.0 was done using West Nile Virus (strain NY2001-6263) provided by ZeptoMetrix®.

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® WNV RT-PCR Kit 2.0 is defined as the concentration (copies/ml) of WNV specific RNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of WNV in human EDTA plasma. West Nile virus material (strain NY2001-6263) was provided by ZeptoMetrix®.

Each dilution was tested in 8 replicates on 3 different days (total n = 24 per dilution) using combinations of 3 RealStar® WNV RT-PCR Kit 2.0 lots, 3 AltoStar® Purification Kit 1.5 lots and 3 AltoStar® Internal Control 1.5 lots. Runs were performed using 3 different AltoStar® Automation System AM16 and CFX96™ Deep Well Real-Time PCR Detection System instruments.

Data from all runs were combined and a probit analysis was performed to determine the 95 % LoD value.

Table 1: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of WNV specific RNA

Input Conc. [copies/ml]	Number of Replicates	Number of Positives	Hit Rate [%]
3.16E+03	24	24	100
1.00E+03	24	24	100
3.16E+02	24	24	100
1.00E+02	24	19	79
3.16E+01	24	9	38
1.00E+01	24	7	29
3.16E+00	24	1	4
1.00E+00	24	0	0

The analytical sensitivity of the RealStar® WNV RT-PCR Kit 2.0 was determined by probit analysis:

- For the detection of WNV specific RNA, the analytical sensitivity is 258 copies/ml [95% confidence interval (CI): 150 - 599 copies/ml]

11.2 Analytical Specificity

The analytical specificity of the RealStar® WNV RT-PCR Kit 2.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant WNV genotypes will be detected.

The analytical specificity of the RealStar® WNV RT-PCR Kit 2.0 with respect to cross reactivity with other pathogens than WNV was evaluated by testing viruses related to WNV, pathogens causing similar symptoms as an infection with WNV and pathogens likely to be present in patients suffering from a WNV infection.

The RealStar® WNV RT-PCR Kit 2.0 did not cross react with any of the following pathogens:

- Dengue virus 1
- Dengue virus 2
- Dengue virus 3
- Dengue virus 4
- Hepatitis A virus
- Hepatitis C virus
- Hepatitis E virus
- Herpes simplex virus 1
- Herpes simplex virus 2
- Human immunodeficiency virus 1
- Japanese encephalitis virus
- Murray Valley encephalitis virus
- *Neisseria meningitidis*
- St. Louis encephalitis virus
- *Streptococcus pneumoniae*
- Tick Borne encephalitis virus (FSME)
- Usutu virus
- Yellow Fever virus
- Zika virus

The RealStar® WNV RT-PCR Kit 2.0 is able to detect WNV from lineages 1 and 2:

- Lineage 1 (NY99)
- Lineage 2 (Heja, B-956 Uganda, 1986)

CAUTION



In case the sample contains other pathogens than WNV competition with the target amplification or cross-reactivities may occur.

11.3 Precision

Precision of the RealStar® WNV RT-PCR Kit 2.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the 3 analyses.

The variability data are expressed in terms of coefficient of variation based on threshold cycle (C_t) - values. At least 4 replicates per sample were analysed for intra-assay variability, inter-assay and inter-lot variability.

Table 2: Precision data (CV % [C_t values]) for WNV high positive EDTA plasma samples

	WNV High Positive Sample [CV% based on C_t values]
Intra-Assay Variability	0.32 - 0.95
Inter-Assay Variability	0.02 - 0.40
Inter-Lot Variability	1.38
Total Variability	1.21

All samples tested at 3x LoD (low positive samples) were detected positive for WNV.

Table 3: Precision data (CV % [C_t values]) for the Internal Control in WNV negative EDTA plasma samples

	Internal Control
Intra-Assay Variability	0.18 - 0.67
Inter-Assay Variability	0.04 - 0.32
Inter-Lot Variability	0.61
Total Variability	1.52

12. Limitations

- Strict compliance with the Instructions for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

13. Quality Control

In accordance with the Altona Diagnostics GmbH ISO EN 13485-certified Quality Management System, each lot of RealStar® WNV RT-PCR Kit 2.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**

phone: **+49-(0)40-5480676-0**

15. Literature

Versalovic, James, Carroll, Karen C., Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

16. Trademarks and Disclaimers

AltoStar®, RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); CFX96™ (Bio-Rad); FAM™, JOE™ (Life Technologies); LightCycler® (Roche); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gene®, QIAamp®, QIASymphony® (QIAGEN); VERSANT® (Siemens Healthcare); ZeptoMetrix®.

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

















The RealStar® WNV RT-PCR Kit 2.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

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17. Explanation of Symbols

Symbol	Explanation
	<i>In vitro</i> diagnostic medical device
	Batch code
	Cap color
	Catalogue number
	Content
	Number
	Component
	Global trade item number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Note
	Version

Notes:

Notes:

always a drop ahead.

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