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artus® WNV LC RT-PCR Kit Handbook

24 (catalog no. 4509003)

96 (catalog no. 4509005)

Version 1
For research use only. Not for use in diagnostic procedures.
For use with the *LightCycler*® Instrument

4509003, 4509005

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R3

Sample to Insight

QIAGEN

Contents

artus WNV LC RT-PCR Kit

For use with the *LightCycler* Instrument.

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Contents

	Labelling and contents	**Cat. No. 4509003 24 reactions**	**Cat. No. 4509005 96 reactions**
Blue	WNV LC Master	2 x 12 reactions	8 x 12 reactions
Red	WNV LC/TM QS 1¤ 4 x 104 cop/µl	1 x 200 µl	1 x 200 µl
Red	WNV LC/TM QS 2¤ 4 x 103 cop/µl	1 x 200 µl	1 x 200 µl
Red	WNV LC/TM QS 3¤ 4 x 102 cop/µl	1 x 200 µl	1 x 200 µl
Red	WNV LC/TM QS 4¤ 4 x 101 cop/µl	1 x 200 µl	1 x 200 µl
Green	WNV LC IC¤	1 x 1,000 µl	2 x 1,000 µl
White	Water (PCR grade)	1 x 1,000 µl	1 x 1,000 µl

¤* IC = Internal Control; QS = Quantitation Standard

Storage

The components of the artus WNV LC RT-PCR Kit should be stored at –30°C to –15°C and are stable until the expiry date stated on the label. Repeated thawing and freezing (>2x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at > 4°C should not exceed a period of 5 hours.

Additionally Required Materials and Devices

- Disposable powder-free gloves
- RNA isolation kit (see 8.1 RNA Isolation)
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Color Compensation Set (Roche Diagnostics, Cat. No. 2 158 850) for the installation of a Crosstalk Color Compensation file
- LightCycler Capillaries (20 µl)
- LightCycler Cooling Block
- LightCycler Instrument
- LightCycler Capping Tool

General Precautions

The user should always pay attention to the following:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls, and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice or in the *LightCycler* Cooling Block.

Pathogen Information

West Nile virus (WNV) is a member of the family *Flaviviridae* (genus *Flavivirus*). Infected mosquitoes usually bite and infect wild birds - the primary host of the virus - but WNV can also infect horses and other mammals. 80% of all infected humans do not present any WNV-related symptoms. WNV infections of elderly people, children, and immunosuppressed individuals may lead to fatal encephalitis or myocarditis in rare cases.

Principle of Real-Time PCR

Pathogen detection by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e. in real time) allows the detection and quantitation of the accumulating product without having to reopen the reaction tubes after the PCR run (Mackay, 2004).

Product Description

The artus WNV LC RT-PCR Kit constitutes a ready-to-use system for the detection of WNV RNA using polymerase chain reaction (PCR) in the *LightCycler* Instrument. The WNV LC Master contains reagents and enzymes for the reverse transcription and specific amplification of a 72 bp region of the WNV genome, and for the direct detection of the specific amplicon in fluorimeter channel F1 of the *LightCycler* Instrument. In addition, the artus WNV LC RT-PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an Internal Control (IC) in fluorimeter channel F3. External positive controls (WNV LC/TM QS 1 - 4) are supplied, which allow the determination of the pathogen load. For further information, please refer to "Quantitation".

Protocol

RNA Isolation

Various manufacturers offer RNA isolation kits. Sample amounts for the RNA isolation procedure depend on the protocol used. Please carry out the RNA isolation according to the manufacturer's instructions. The following isolation kit is recommended:

Sample material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer	Carrier RNA
Plasma, CSF	QIAamp® Viral RNA Mini Kit (50)	52 904	QIAGEN	Included

1. The use of carrier RNA is critical for the extraction efficiency and, consequently, for DNA/RNA yield. To increase the stability of the carrier RNA provided with the QIAamp Viral RNA Mini Kit, we recommend the following procedure deviant from the user manual of the extraction kit:

   1a. Resuspend the lyophilized carrier RNA prior to first use of the extraction kit in 310 µl AE or AVE buffer (elution buffer, final concentration 1 µg/µl, do not use lysis buffer). Portion this carrier RNA solution into a number of aliquots adequate to your needs and store them at –30 to –15°C. Avoid repeated thawing (>2x) of a carrier RNA aliquot.

1b. Before beginning of each extraction, a mixture of lysis buffer and carrier RNA (and Internal Control, where applicable, see "Internal Control") should be prepared freshly according to the following pipetting scheme:

Number of samples	**1**	**12**
Lysis buffer AVL	560 µl	6,720 µl
Carrier RNA (1 µg/µl)	5.6 µl	67.2 µl
Total volume	**565.6 µl**	**6,787.2 µl**
Volume per extraction	**560 µl**	**each 560 µl**

1c. Please use the freshly prepared lysis buffer instantly for extraction. Storage of the mixture is not possible!

2. When using isolation protocols with ethanol-containing washing buffers, carry out an additional centrifugation step (three minutes, 13,000 rpm) before the elution to remove any remaining ethanol. This prevents possible inhibition of PCR.
3. The *artus* WNV LC RT-PCR Kit should not be used with **phenol**-based isolation methods.

Important: The Internal Control of the *artus* WNV LC RT-PCR Kit can be used directly in the isolation procedure (see "Internal Control").

Internal Control

An *Internal Control* (*WNV LC IC*) is supplied. This allows the user **both to control the RNA isolation procedure and to check for possible PCR inhibition** (see Figure. 1). For this application, add the Internal Control to the isolation at a ratio of 0.1 µl per 1 µl elution volume. For example, using the QIAmp Viral RNA Mini Kit, the RNA is eluted in 60 µl AVE buffer. Hence, 6 µl of the *Internal Control* should be added initially. If you elute e.g. in 50 µl, then use the corresponding volume of 5 µl. The quantity of *Internal Control* used depends **only** on the elution volume. Please note that the *Internal Control* should be added to the mixture of lysis buffer and sample material. Alternatively, the *Internal Control* can be added directly to the lysis buffer. Optionally, you can add the carrier RNA together with the *Internal Control* to the lysis buffer (see "RNA Isolation"). However, please note that the mixture of Internal

Control/carrier RNA and lysis buffer has to be prepared freshly and used instantly (storage of the mixture at room temperature or in the fridge for only a few hours may lead to *Internal Control* failure and a reduced extraction efficiency). Please do not add the *Internal Control* to the sample material directly!

The *Internal Control* can optionally be used exclusively to check for possible PCR inhibition (see Figure. 2). For this application, add 0.5 µl of the *Internal Control* per reaction directly to 15 µl WNV LC Master. For each PCR reaction, use 15 µl of the Master Mix produced as described below*, and add 5 µl of the purified sample. If you are preparing a PCR run for several samples, please increase the volume of the WNV LC Master and the *Internal Control* according to the number of samples (see "Preparing the PCR").

Quantitation

The enclosed *Quantitation Standards* (*WNV LC/TM QS 1 - 4*) are treated as previously purified samples and the same volume is used (5 µl). To generate a standard curve on the *LightCycler* Instrument, all four *Quantitation Standards* should be used and defined in the *Sample Loading Screen* as standards with the specified concentrations (see *LightCycler Operator's Manual*, Version 3.5, Chapter B, 2.4. Sample Data Entry). The standard curve generated as above can also be used for subsequent runs, provided that at least one standard of one given concentration is used in the current run. For this purpose, the previously generated standard curve needs to be imported (see *LightCycler Operator's Manual*, Version 3.5, Chapter B, 4.2.5. Quantitation with an External Standard Curve). However, this quantitation method may lead to deviations in the results due to variability between different PCR runs.

Attention: The *Quantitation Standards* are defined as copies/µl. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material:

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/µl) x Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

Please note that as a matter of principle, the **initial** sample volume should be entered in the equation above. This has to be considered when the sample volume has been changed prior to the nucleic acid extraction (e.g. narrowing the volume by centrifugation or increase of volume by replenishment to the volume required for the isolation).

* The volume increase caused by adding the *Internal Control* is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

Important: A guideline for the quantitative analysis of *artus* systems on the *LightCycler* Instrument is provided at **www.qiagen.com/TechnicalNoteLightCycler2**.

Preparing the PCR

Make sure that the Cooling Block as well as the capillary adapters (accessories of the Instrument) are pre-cooled to 4°C. Place the desired number of *LightCycler* capillaries into the adapters of the Cooling Block. Please make sure that at least one *Quantitation Standard* as well as one negative control (*Water, PCR grade*) are included per PCR run. To generate a standard curve, use all supplied Quantitation Standards (WNV LC/TM QS 1 - 4) for each PCR run. Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by inverting the tube several times), and centrifuged briefly.

If you want to use the *Internal Control* **to monitor the RNA isolation procedure and to check for possible PCR inhibition**, it has already been added to the isolation (see "Internal Control"). In this case, please use the following pipetting scheme (for a schematic overview, see Figure. 1):

	Number of samples	1	12
Preparation of Master Mix	*WNV LC Master*	15 µl	180 µl
	WNV LC IC	0 µl	0 µl
	Total volume	15 µl	180 µl
Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample	5 µl	5 µl each
	Total volume	20 µl	20 µl each

If you want to use the *Internal Control* **exclusively to check for PCR inhibition**, it must be added directly to the *WNV LC Master*. In this case, please use the following pipetting scheme (for a schematic overview, see Figure. 2):

	Number of samples	1	12
Preparation of Master Mix	*WNV LC Master*	15 µl	180 µl
	WNV LC IC	0.5 µl	6 µl
	Total volume	15.5 µl	186 µl
Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample	5 µl	5 µl each
	Total volume	20 µl	20 µl each

Pipette 15 µl of the Master Mix into the plastic reservoir of each capillary. Then add 5 µl of the eluted sample RNA. Correspondingly, 5 µl of at least one of the *Quantitation Standards* (*WNV LC/TM QS 1 - 4*) must be used as a positive control and 5 µl of water (*Water, PCR grade*) as a negative control. Close the capillaries. To transfer the mixture from the plastic reservoir into the capillary, centrifuge the adapters containing the capillaries in a desktop centrifuge for ten seconds at a maximum of 400 x *g* (2,000 rpm).

* The volume increase caused by adding the *Internal Control* is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

Addition of the *Internal Control* to the Purification Procedure

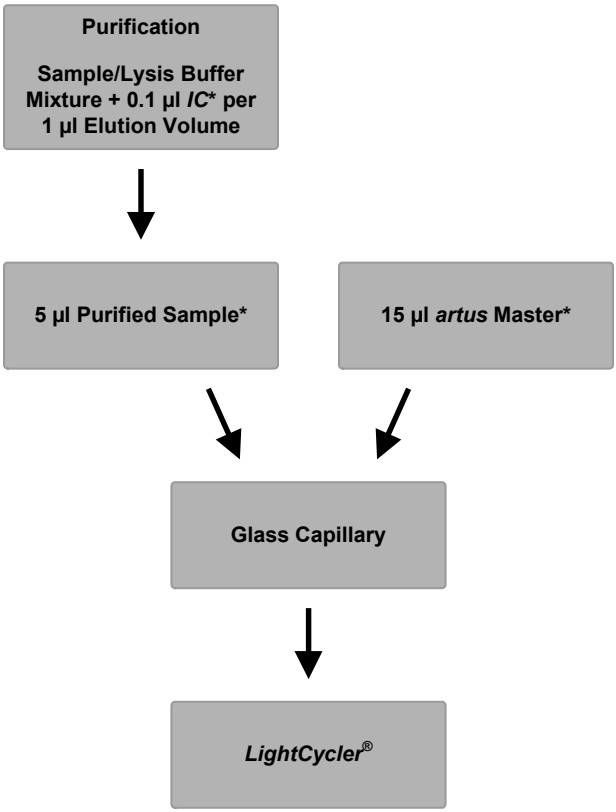

Figure. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition. *Please make sure that the solutions are thawed completely, mixed well, and centrifuged briefly.

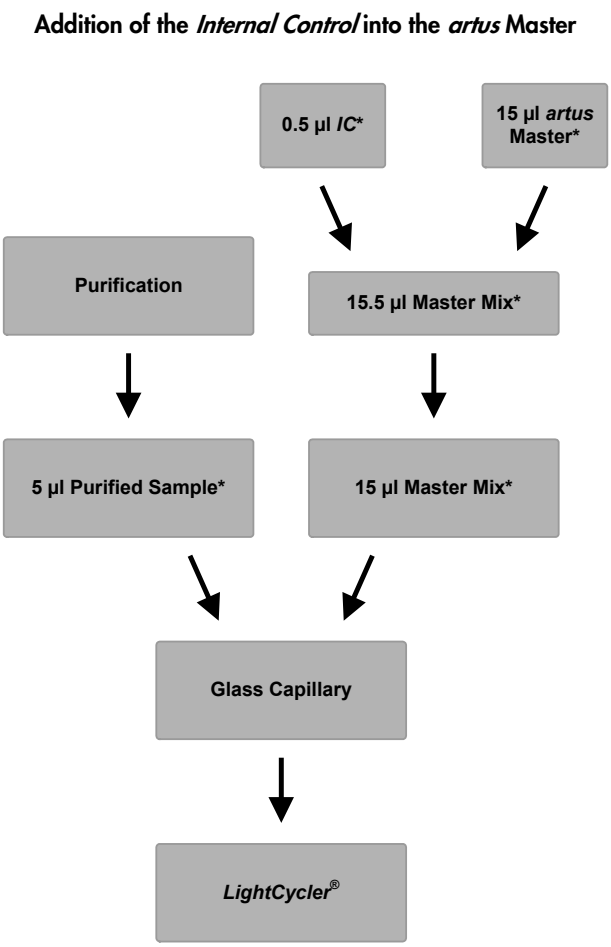

Figure. 2: Schematic workflow for the control of PCR inhibition.
***Please make sure that the solutions are thawed completely, mixed well, and centrifuged briefly.**

Programming of the *LightCycler* Instrument

For the detection of WNV RNA, create a temperature profile on your *LightCycler* Instrument according to the following four steps (see Figures 3 - 6).

1. Reverse Transcription of the RNA — Fig. 3
2. Initial Activation of the Hot Start Enzyme — Fig. 4
3. Amplification of the cDNA — Fig. 5
4. Cooling — Fig. 6

Pay particular attention to the settings for *Analysis Mode*, *Cycle Program Data*, and *Temperature Targets*. In the illustrations, these settings are framed in bold black. Please find further information on programming the *LightCycler* Instrument in the *LightCycler Operator's Manual*.

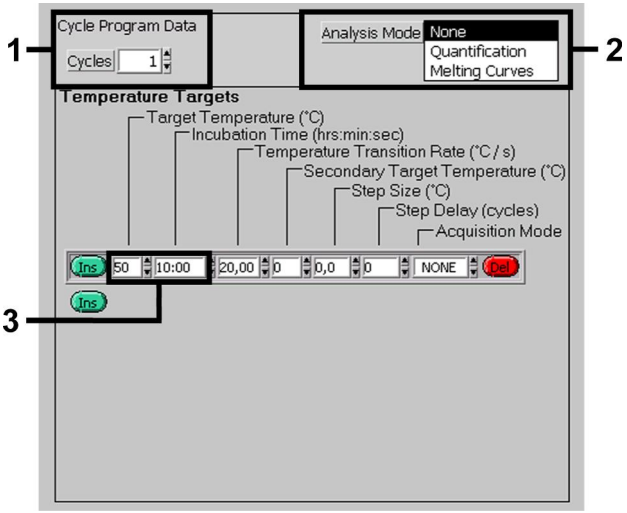

Figure 3. Reverse Transcription of the RNA.

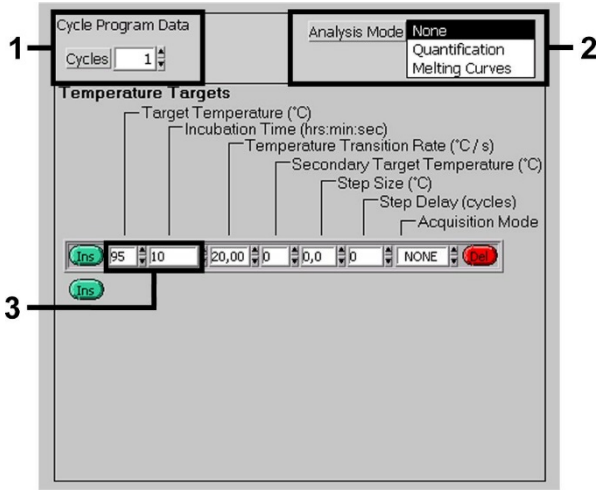

Figure. 3. Initial Activation of the Hot Start Enzyme.

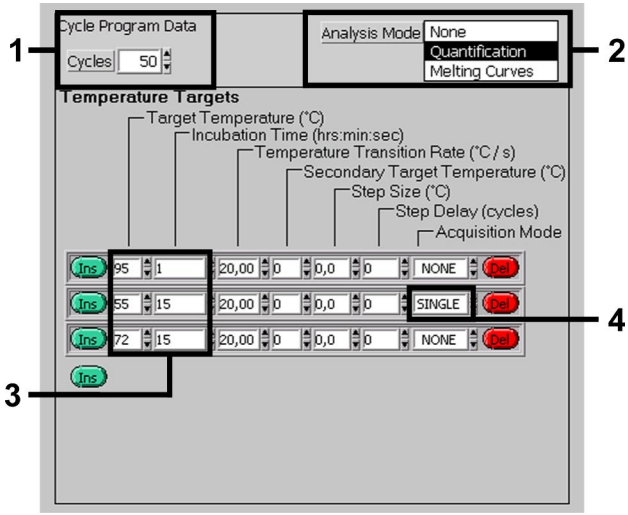

Figure 4. Amplification of the cDNA.

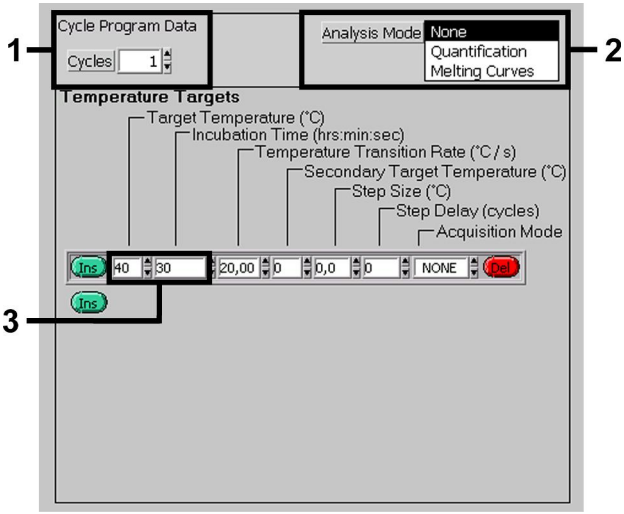

Figure 5. Cooling.

Data Analysis

In multicolor analyses, interferences occur between fluorimeter channels. The *LightCycler* Instrument's software contains a file termed *Color Compensation File*, which compensates for these interferences. Open this file before, during or after the PCR run by activating the *Choose CCC File* or the *Select CC Data* button. If no *Color Compensation File* is installed, generate the file according to the instructions in the *LightCycler Operator's Manual*. After the *Color Compensation File* has been activated, separate signals appear in fluorimeter channels F1, F2 and F3. For analysis of the PCR results gained with the *artus* WNV LC RT-PCR Kit, please select fluorescence display options F1 for the analytical WNV RT-PCR and F3/Back-F1* for the *Internal Control* RT-PCR. For the analysis of quantitative runs, please follow the instructions given in 8.3 Quantitation and in the **Technical Note for quantitation on the *LightCycler* Instrument** at **www.qiagen.com/TechnicalNoteLightCycler2**.

* When using older software versions (version 3.3 and older) the display option F3/Back-F1 is not available. In this case select F3/F1.

The following results are possible:

- A signal is detected in fluorimeter channel F1.

  The result of the analysis is positive: The sample contains WNV RNA.

  In this case, the detection of a signal in the F3/Back-F1 channel is dispensable, since high initial concentrations of WNV RNA (positive signal in the F1 channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the F3/Back-F1 channel (competition).

  In fluorimeter channel F1, no signal is detected. At the same time, a signal from the *Internal Control* appears in the F3/Back-F1 channel.

  The result of the analysis is negative: No WNV RNA is detectable in the sample.

  In the case of a negative WNV RT-PCR, the detected signal of the *Internal Control* rules out the possibility of PCR inhibition.

* When using older software versions (version 3.3 and older), the display option F3/Back-F1 is not available. In this case, select F3/F1.

- No signal is detected in the F1 or in the F3/Back-F1 channel.

  No result can be concluded.

  Information regarding error sources and their solution can be found in Troubleshooting.

Examples of positive and negative PCR reactions are given in Figure 7 and Figure 8, respectively.

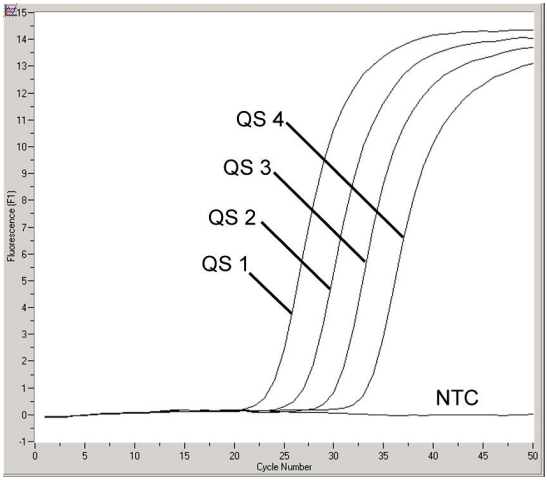

Figure 6. Detection of the *Quantitation Standards* (*WNV LC/TM QS 1 - 4*) in fluorimeter channel F1. NTC: non-template control (negative control).

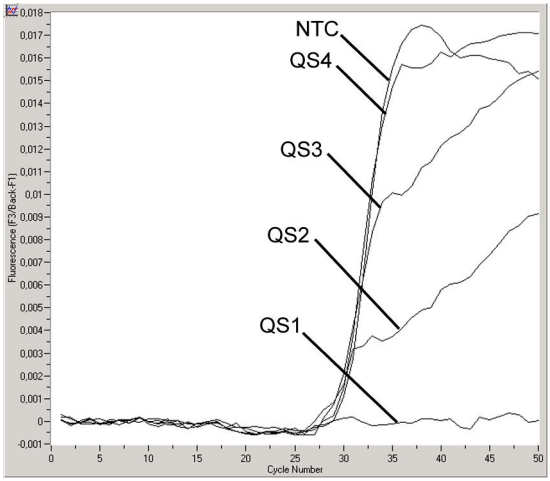

Figure 7. Detection of the *Internal Control* (*IC*) in fluorimeter channel F3/Back-F1 with simultaneous amplification of *Quantitation Standards* (*WNV LC/TM QS 1 - 4*). NTC: non-template control (negative control).

Troubleshooting

No signal with positive controls (*WNV LC/TM QS 1 - 4*) in fluorimeter channel F1:

- The selected fluorimeter channel for PCR data analysis does not comply with the protocol.
  - For data analysis, select the fluorimeter channel F1 for the analytical WNV RT-PCR and the fluorimeter channel F3/Back-F1 for the *Internal Control* RT-PCR.
- Incorrect programming of the temperature profile of the *LightCycler* Instrument.
  - Compare the temperature profile with the protocol (see **8.5 Programming of the *LightCycler* Instrument**).
- Incorrect configuration of the PCR reaction.
  - Check your work steps by means of the pipetting scheme (see **8.4 Preparing the PCR**) and repeat the PCR, if necessary.
- The storage conditions for one or more kit components did not comply with the instructions given in **2. Storage** or the *artus* WNV LC RT-PCR Kit had expired.
  - Please check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Weak or no signal of the Internal Control in fluorimeter channel F3/Back F1 and simultaneous absence of a signal in channel F1:

- The PCR conditions do not comply with the protocol.
  - Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
- The PCR was inhibited.
  - Make sure that you use a recommended isolation method (see **8.1 RNA Isolation**) and stick closely to the manufacturer's instructions.
  - Make sure that during the RNA isolation, the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see **8.1 RNA Isolation**).

- RNA was lost during extraction.
  - If the *Internal Control* had been added to the extraction, an absent signal of the *Internal Control* can indicate the loss of RNA during the extraction. Make sure that you use a recommended isolation method (see **8.1 RNA Isolation**) and stick closely to the manufacturer's instructions.
- The storage conditions for one or more kit components did not comply with the instructions given in **2. Storage**, or the *artus* WNV LC RT-PCR Kit had expired.
  - Please check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Signals with the negative controls in fluorimeter channel F1 of the analytical PCR

- A contamination occurred during preparation of the PCR.
  - Repeat the PCR with new reagents in replicates.
  - If possible, close the PCR tubes directly after addition of the sample to be tested.
  - Strictly pipette the positive controls at last.
  - Make sure that work space and instruments are decontaminated at regular intervals.
- A contamination occurred during extraction.
  - Repeat the extraction and PCR of the sample to be tested using new reagents.
  - Make sure that work space and instruments are decontaminated at regular intervals.

If you have any further questions or if you encounter problems, please contact our Technical Service.

Product Use Limitations

- The *artus* WNV LC RT-PCR Kit is for research use only. Not for use in diagnostic procedures.
- No claim or representation is intended for their use for a specific clinical use (diagnostic, prognostic, or therapeutic).
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

Warnings and Precautions

For safety information of the *artus* WNV LC RT-PCR Kit, please consult the appropriate safety data sheet (SDS). The SDSs are available online in convenient and compact PDF format at **www.qiagen.com/safety**.

Quality Control

In accordance with QIAGEN's ISO 9001 and ISO 13485-certified Quality Management System, each lot of *artus* WNV LC RT-PCR Kit has been tested against predetermined specifications to ensure consistent product quality.

References

Mackay IM. Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. 2004; **10** (3): 190 - 212.

Symbols

Use by

Batch code

Manufacturer

Catalog number

Material number

Global Trade Item Number

Contains sufficient for <N> tests

Temperature limitation

Quantitation Standard

QS

Internal Control

IC

Ordering Information

Product	Contents	Cat. no.
artus WNV LC RT-PCR Kit (24)	For 24 reactions: Master, 4 Quantification Standards, Internal Control, Water (PCR Grade)	4509003
artus WNV LC RT-PCR Kit (96)	For 24 reactions: Master, 4 Quantification Standards, Internal Control, Water (PCR Grade)	4509005
QIAamp Viral RNA Mini Kit	For 50 minipreps: 50 QIAamp Mini Spin Columns, Carrier RNA, Buffers an Collection Tubes (2 ml)	52904

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
R3 12/2019	Removal of text referring to Performance Characteristics. Updated list of sample materials, steps in preparing the PCR, and product use limitation. Layout updates.

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