artus® WNV LC RT-PCR Kit

Handbook

Σ 24

Quantitative in vitro Diagnostics

For use with the *LightCycler*® Instrument

December 2014 — Version 1

CE

IVD

REF 4509063, 4509065

1046924EN

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

R3 MAT 1046924EN

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Table of Contents

artus WNV LC RT-PCR Kit

For use with the *LightCycler* Instrument.

1. Contents

	Labeling and contents	Art. No. 4509063 24 reactions
Blue	*WNV LC Master*	2 x 12 reactions
Red	*WNV LC/TM QS 1*¤ *4 x 10*^{4} *copies/µl*	1 x 200 µl
Red	*WNV LC/TM QS 2*¤ *4 x 10*^{3} *copies/µl*	1 x 200 µl
Red	*WNV LC/TM QS 3*¤ *4 x 10*^{2} *copies/µl*	1 x 200 µl
Red	*WNV LC/TM QS 4*¤ *4 x 10*^{1} *copies/µl*	1 x 200 µl
Green	*WNV LC IC*¤	1 x 1000 µl
White	*Water* (*PCR grade*)	1 x 1000 µl

¤ *QS* = *Quantitation Standard*
IC = *Internal Control*

2. Storage

The components of the *artus* WNV LC RT-PCR Kit should be stored at –15°C to –30°C and are stable until the expiry date stated on the label. Repeated thawing and freezing (> 2 x) 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 five hours.

3. Additionally Required Materials and Devices

- Disposable powder-free gloves
- RNA isolation kit (see **8.1 RNA Isolation**)
- Pipets (adjustable)
- Sterile pipet tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- *Color Compensation Set* (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

4. General Precautions

The user should always pay attention to the following:

- Use sterile pipet 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.

5. 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 patients may in rare cases lead to fatal encephalitis or myocarditis.

6. Principle of Real-Time PCR

Pathogen diagnosis 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 which 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 re-open the reaction tubes after the PCR run (Mackay, 2004).

7. 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. The detection limit of the analytical WNV RT-PCR (see **11.1 Analytical Sensitivity**) is not reduced. 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 section **8.3 Quantitation**.

8. Protocol

8.1 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	Catalog Number	Manufacturer	Carrier RNA
Serum, Plasma, CSF	QIAamp® Viral RNA Mini Kit (50)	52 904	QIAGEN	included

- 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:
  a. Resuspend the lyophilized carrier RNA prior to first use of the extraction kit in 310 µl Buffer AE or Buffer AVE (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 –20°C. Avoid repeated thawing (> 2 x) of a carrier RNA aliquot.
  b. Before beginning of each extraction, a mixture of lysis buffer and carrier RNA (and *Internal Control*, where applicable, see **8.2 Internal Control**) should be prepared freshly according to the following pipetting scheme:

Number of samples	1	12
Buffer AVL	560 µl	6720 µl
Carrier RNA (1 µg/µl)	5.6 µl	67.2 µl
Total volume	**565.6 µl**	**6787.2 µl**
Volume per extraction	**560 µl**	**560 µl each**

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

- When using isolation protocols with **ethanol**-containing washing buffers, please 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.
- 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 **8.2 Internal Control**).

8.2 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 Fig. 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 Buffer AVE. 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 **8.1 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 Fig. 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 above* 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 **8.4 Preparing the PCR**).

8.3 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:

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

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/Products/ByLabFocus/MDX** (**Technical Note for quantitation on the *LightCycler* Instrument**).

8.4 Preparing the PCR

Make sure that the Cooling Block as well as the capillary adapters (accessories of the *LightCycler* 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 **8.2 Internal Control**). In this case, please use the following pipetting scheme (for a schematic overview see Fig. 1):

	Number of samples	**1**	**12**
1. Preparation of Master Mix	*WNV LC Master*	15 µl	180 µl
	WNV LC IC	0 µl	0 µl
	Total volume	**15 µl**	**180 µl**
2. 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 Fig. 2):

	Number of samples	1	12
1. 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**
2. Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample	5 µl	5 µl each
	Total volume	**20 µl**	**20 µl each**

Pipet 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* (2000 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

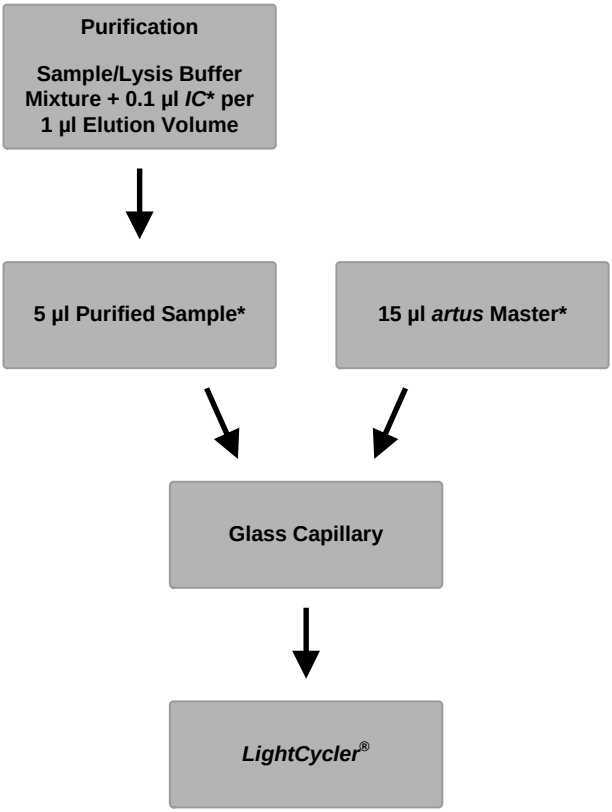


Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

*Please make sure that the solutions are thawed completely, mixed well and centrifuged briefly.

Addition of the *Internal Control* into the *artus* Master

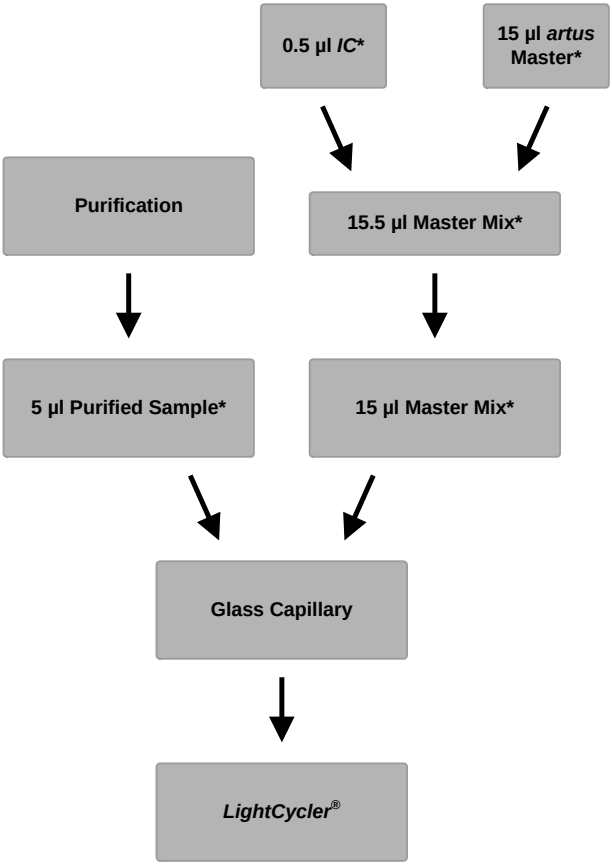


Fig. 2: Schematic workflow for the control of PCR inhibition.

*Please make sure that the solutions are thawed completely, mixed well and centrifuged briefly.

8.5 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 Fig. 3 – 6).

A.	Reverse Transcription of the RNA	Fig. 3
B.	Initial Activation of the Hot Start Enzyme	Fig. 4
C.	Amplification of the cDNA	Fig. 5
D.	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*.

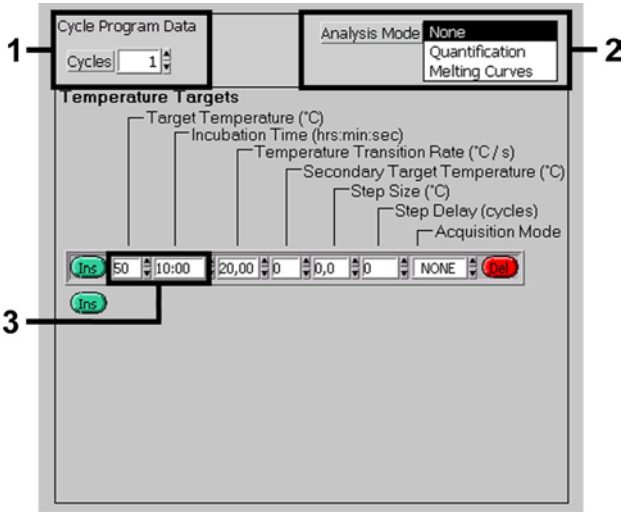


Fig. 3: Reverse Transcription of the RNA.

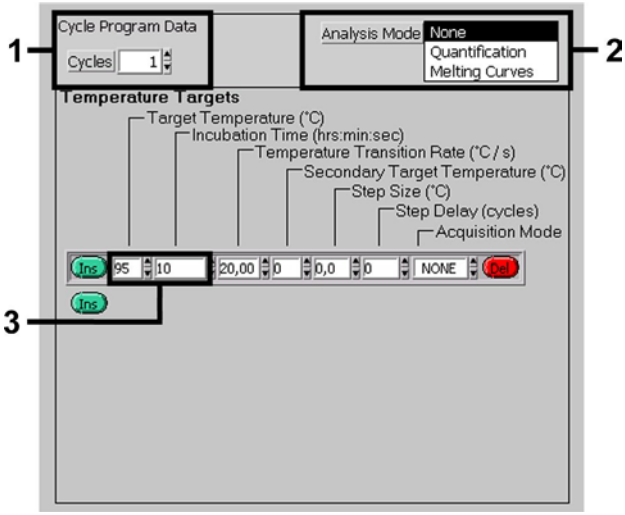


Fig. 4: Initial Activation of the Hot Start Enzyme.

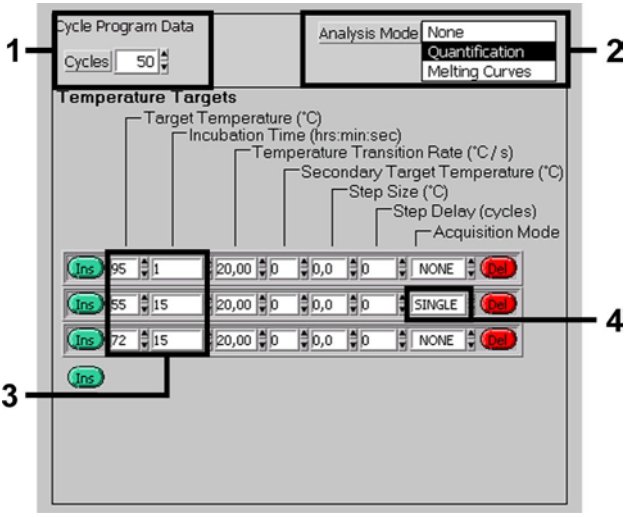


Fig. 5: Amplification of the cDNA.

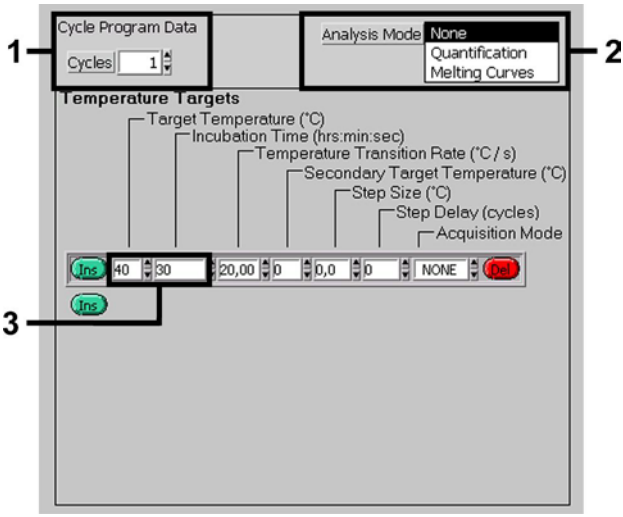


Fig. 6: Cooling.

9. 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, respectively. For the analysis of quantitative runs, please follow the instructions given in **8.3 Quantitation** and in the **Technical Note for**

* 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.

quantitation on the *LightCycler* Instrument at **www.qiagen.com/Products/ByLabFocus/MDX**.

The following results are possible:

1. 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).

2. 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.

   In the sample no WNV RNA is detectable. It can be considered negative.

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

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

   No diagnosis can be concluded.

   Information regarding error sources and their solution can be found in **10. Troubleshooting**.

Examples of positive and negative PCR reactions are given in Fig. 7 and Fig. 8.

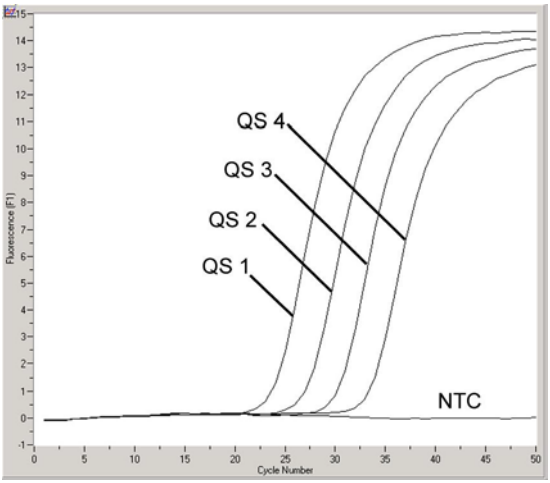


Fig. 7: Detection of the *Quantitation Standards* (*WNV LC/TM QS 1 – 4*) in fluorimeter channel F1. NTC: no template control (negative control).

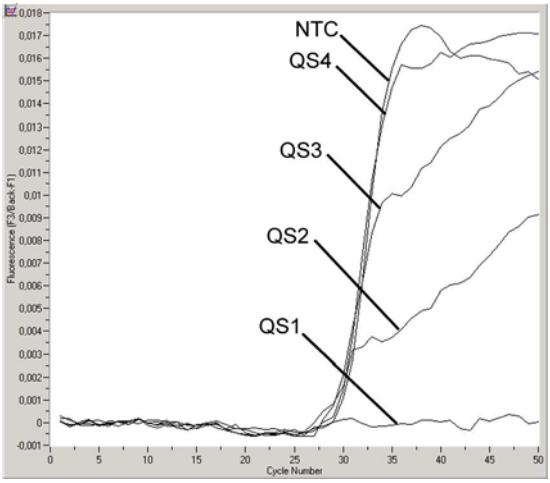


Fig. 8: Detection of the *Internal Control* (*IC*) in fluorimeter channel F3/Back-F1 with simultaneous amplification of *Quantitation Standards* (*WNV LC/TM QS 1 – 4*). NTC: no template control (negative control).

10. 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* 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 pipet the positive controls 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.

11. Specifications

11.1 Analytical Sensitivity

In order to determine the analytical sensitivity of the *artus* WNV LC RT-PCR Kit, a standard dilution series has been set up from 40 to nominal 0.01265 of in vitro transcribed RNA copies per microliter of the WNV amplicon and analyzed with the *artus* WNV LC RT-PCR Kit. Testing was carried out on three different days on eight replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Fig. 9. The detection limit of the *artus* WNV LC RT-PCR Kit is 2.4 copies/µl (p = 0.05). This means that there is a 95 % probability that 2.4 copies/µl will be detected.

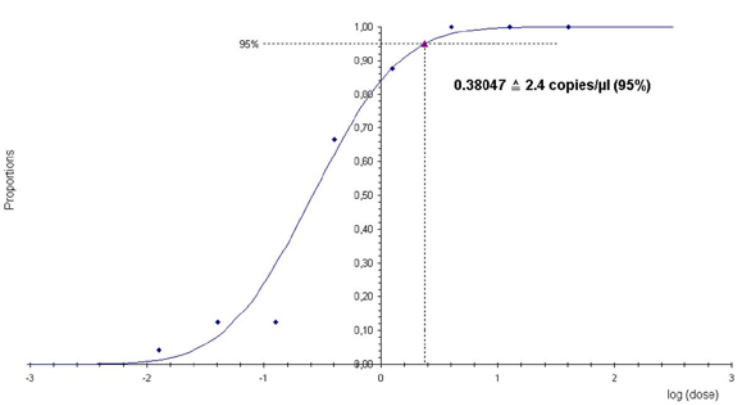


Fig. 9: Analytical sensitivity of the *artus* WNV LC RT-PCR Kit.

11.2 Specificity

The specificity of the *artus* WNV LC RT-PCR Kit is first and foremost ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence

comparison analysis. The detectability of all relevant WNV strains has thus been ensured by a database alignment.

Additionally, the influence of genomic DNA on the detection of WNV positive samples has been tested. It has been shown that large amounts of genomic DNA in a PCR run can inhibit the PCR reaction. Therefore, the *artus* WNV LC RT-PCR Kit should only be used with cell-poor sample materials.

Moreover, the specificity was validated with 30 different WNV negative plasma and cerebrospinal fluid samples. These did not generate any signals with the WNV specific primers and probes, which are included in the *WNV LC Master*.

To determine the specificity of the *artus* WNV LC RT-PCR Kit the control group listed in the following table (see Table 1) has been tested for cross-reactivity. None of the tested pathogens has been reactive.

Table 1: Testing the specificity of the kit with potentially cross-reactive pathogens.

Control group	WNV (F1)	*Internal Control* (F3/Back-F1)
St. Louis encephalitis virus	–	+
Japanese encephalitis virus	–	+
Yellow fever virus	–	+
Dengue virus type 1	–	+
Dengue virus type 2	–	+
Dengue virus type 3	–	+
Dengue virus type 4	–	+
Montana myotis leukoencephalitis virus	–	+
Modoc virus	–	+
Human herpesvirus 1 (Herpes simplex virus 1)	–	+
Human herpesvirus 2 (Herpes simplex virus 2)	–	+
Human herpesvirus 3 (Varicella-zoster virus)	–	+
Human herpesvirus 5 (Cytomegalovirus)	–	+
Human immunodeficiency virus	–	+
Enterovirus 71	–	+
Coxsackievirus A7	–	+
Coxsackievirus A24	–	+
Coxsackievirus B3	–	+
Echovirus 30	–	+
Hepatitis A virus	–	+
Hepatitis B virus	–	+
Hepatitis C virus	–	+
Mycobacterium tuberculosis	–	+
Plasmodium falciparum	–	+
Listeria welshmerii	–	+
Listeria ivanovii	–	+

11.3 Precision

The precision data of the *artus* WNV LC RT-PCR Kit allow the determination of the total variance of the assay. The total variance consists of the **intra-assay variability** (variability of multiple results of samples of the same concentration within one experiment), the **inter-assay variability** (variability of

multiple results of the assay generated on different instruments of the same type by different operators within one laboratory) and the **inter-batch variability** (variability of multiple results of the assay using various batches). The data obtained were used to determine the standard deviation, the variance and the coefficient of variation for the pathogen specific and the *Internal Control* PCR.

Precision data of the *artus* WNV LC RT-PCR Kit have been collected using the *Quantitation Standard* of the lowest concentration (*QS 4*; 40 copies/µl). Testing was performed with eight replicates. The precision data were calculated on basis of the Ct values of the amplification curves (Ct: threshold cycle, see Table 2). In addition, precision data for quantitative results in copies/µl were determined using the corresponding Ct values (see Table 3). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 0.79 % (Ct) or 10.12 % (conc.), for the detection of the *Internal Control* 4.28 % (Ct). These values are based on the totality of all single values of the determined variabilities.

Table 2: Precision data on basis of the Ct values.

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: *WNV LC/TM QS 4*	0.07	0.01	0.22
Intra-assay variability: *Internal Control*	0.14	0.02	0.46
Inter-assay variability: *WNV LC/TM QS 4*	0.30	0.09	0.91
Inter-assay variability: *Internal Control*	0.32	0.10	1.09
Inter-batch variability: *WNV LC/TM QS 4*	0.13	0.02	0.40
Inter-batch variability: *Internal Control*	1.28	1.63	4.62
Total variance: *WNV LC/TM QS 4*	0.26	0.07	0.79
Total variance: *Internal Control*	1.23	1.51	4.28

Table 3: Precision data on basis of the quantitative results (in copies/µl).

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: *WNV LC/TM QS 4*	2.16	4.65	5.38
Inter-assay variability: *WNV LC/TM QS 4*	5.63	31.73	13.95
Inter-batch variability: *WNV LC/TM QS 4*	2.53	6.40	6.31
Total variance: *WNV LC/TM QS 4*	4.07	16.56	10.12

11.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* WNV LC RT-PCR Kit. 30 WNV negative samples of plasma and cerebrospinal fluid were spiked with 7.2 copies/µl elution volume of West Nile virus full-length RNA (threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp Viral RNA Mini Kit (see **8.1 RNA Isolation**) these samples were analyzed with the *artus* WNV LC RT-PCR Kit. For all WNV samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 30 WNV negative plasma and cereprospinal fluid samples. The total failure rate was 0 %. Inhibitions were not observed. Thus, the robustness of the *artus* WNV LC RT-PCR Kit is ≥ 99 %.

11.5 Reproducibility

Up-to-date interlaboratory tests for the real-time PCR detection of West Nile virus RNA are not available. Reproducibility data will be collected in external validation and beta-side studies and in comparison with other products in diagnostic studies (see **11.6 Diagnostic Evaluation**).

11.6 Diagnostic Evaluation

Currently, the *artus* WNV LC RT-PCR Kit is undergoing a series of evaluation studies.

12. Product Use Limitations

- All reagents may exclusively be used in in vitro diagnostics.
- The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only.
- 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.
- Although rare, mutations within the highly conserved regions of the viral genome covered by the kit's primers and/or probe may result in underquantitation or failure to detect the presence of the virus in these cases. Validity and performance of the assay design are revised at regular intervals.

13. Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN® kit and kit component.

Discard sample and assay waste according to your local safety regulations.

14. Quality Control

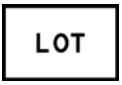
In accordance with QIAGEN's Total Quality Management System, each lot of *artus* WNV LC RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

15. References

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

16. Explanation of Symbols

Use by



Batch code

Manufacturer

Catalog number

Material number

Handbook

In vitro diagnostic medical device

Components

Contains

Number

Global Trade Item Number

<N>

Contains sufficient for <N> tests

Temperature limitation

Consult instructions for use

QS *Quantitation Standard*

IC *Internal Control*

This page intentionally left blank

artus WNV LC RT-PCR Kit

Trademarks and Disclaimers
QIAGEN®, QIAamp®, *artus*® (QIAGEN Group); *LightCycler*® (Roche Diagnostics).

The purchase of this product allows the purchaser to use it for the performance of diagnostic services for human in vitro diagnostics. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

THE PURCHASE OF THIS PRODUCT GRANTS THE PURCHASER RIGHTS UNDER ONE OR MORE OF U.S. PATENT NOS 6,174,670, 7,160,998, 6,569,627 AND 6,245,514 AND THEIR FOREIGN COUNTERPARTS TO USE THIS PRODUCT SOLELY FOR PROVIDING HUMAN AND ANIMAL IN VITRO DIAGNOSTIC SERVICES. NO GENERAL PATENT OR OTHER LICENSE OF ANY KIND OTHER THAN THIS SPECIFIC RIGHT OF USE FROM PURCHASE IS GRANTED HEREBY.

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the *artus* WNV LC RT-PCR Kit to the following terms:

1. The *artus* WNV LC RT-PCR Kit may be used solely in accordance with the artus *WNV LC RT-PCR Kit Handbook* and for use with components contained in the Kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the artus *WNV LC RT-PCR Kit Handbook* and additional protocols available at www.qiagen.com.

2. Other than expressly stated licenses, QIAGEN makes no warranty that this Kit and/or its use(s) do not infringe the rights of third-parties.

3. This Kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of the Kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the Kit and/or its components.

For updated license terms, see www.qiagen.com.

QIAamp Viral RNA Mini Kit

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.

www.qiagen.com

Australia ■ Orders 1-800-243-800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ Orders 0800-28-10-10 ■ Fax 0800-28-10-19 ■ Technical 0800-28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 86-21-3865-3865 ■ Fax 86-21-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 800-789-544 ■ Fax 02-334304-826 ■ Technical 800-787980

Japan ■ Telephone 03-6890-7300 ■ Fax 03-5547-0818 ■ Technical 03-6890-7300

Korea (South) ■ Orders 080-000-7146 ■ Fax 02-2626-5703 ■ Technical 080-000-7145

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

Mexico ■ Orders 01-800-7742-639 ■ Fax 01-800-1122-330 ■ Technical 01-800-7742-436

The Netherlands ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Norway ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

Singapore ■ Orders 1800-742-4362 ■ Fax 65-6854-8184 ■ Technical 1800-742-4368

Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

1046924 148051765

Sample & Assay Technologies