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artus® SARS RG RT-PCR Kit Handbook

Σ 24 (catalog no. 4511263)

Quantitative in vitro Diagnostics

For use with the *artus 3000* and the *Rotor-Gene*® *3000*

Version 1

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IVD

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Contents

artus SARS RG RT-PCR Kit

For use with the *artus 3000* or *Rotor-Gene 3000**.

1. Contents

	Labelling and contents	Art. No. 4511263 24 reactions
Blue	*SARS-CoV RG/TM Master*	2 x 12 rxns
Red	*SARS-CoV LC/RG/TM QS 1*¤ *1 x 10^4 cop/µl*	1 x 200 µl
Red	*SARS-CoV LC/RG/TM QS 2*¤ *1 x 10^3 cop/µl*	1 x 200 µl
Red	*SARS-CoV LC/RG/TM QS 3*¤ *1 x 10^2 cop/µl*	1 x 200 µl
Red	*SARS-CoV LC/RG/TM QS 4*¤ *1 x 10^1 cop/µl*	1 x 200 µl
Green	*SARS-CoV LC/RG/TM IC*¤	1 x 1,000 µl
White	*Water (PCR grade)*	1 x 1,000 µl

¤ *QS = Quantitation Standard*
IC = Internal Control

2. Storage

The components of the *artus* SARS RG 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 8.2 RNA Isolation)
- Pipettes (adjustable)

* The artus SARS RG RT-PCR Kit can be used with the Rotor-Gene™ *2000* as well.

- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- *artus 3000* or *Rotor-Gene 3000*
- 0.1 ml PCR tubes for use with 72-well rotor (0.1 ml Strip Tubes and Caps, QIAGEN Hamburg, Cat. No.: 4699982; 0.1 ml tubes, Corbett Research, Cat. No.: ST-1001)
- Alternatively: 0.2 ml PCR tubes for use with 36-well rotor (e.g. 0.2 ml PCR Tubes, QIAGEN Hamburg, Cat. No.: 4699983; 0.2 ml tubes, Corbett Research, Cat. No.: SE-1003F)
- Cooling Block (72-/96-Well Loading Block, QIAGEN Hamburg, Cat. No.: 4699980/4699981; 72/96 well loading block, Corbett Research, Cat. No.: 3001-008/3001-009)

4. 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 Cooling Block (72/96 well loading block).

5. Pathogen Information

Coronaviruses, a genus in the family *Coronaviridae*, are large enveloped, positive-stranded RNA viruses that cause highly virulent disease in humans and domestic animals. Two coronaviruses known to infect humans account for one third of common colds and are also a well-known cause of nosocomial upper respiratory infections in premature infants.

A novel member of the coronavirus family is considered to be the causative agent of the Severe Acute Respiratory Syndrome (SARS). A part of a polymerase gene of the SARS-Coronavirus (SARS-CoV) was identified via PCR in a SARS patient by the Bernhard-Nocht-Institute for Tropical Medicine in Hamburg and at cooperating laboratories. This assay was used to establish a commercially available real-time RT-PCR system for the direct detection of the SARS-CoV

species. PCR can detect genetic material of the SARS-CoV in various specimens (blood, respiratory secretions or body tissues).

Interpretation of test results

Attention: Please consider the official recommendations of the World Health Organization (WHO) that can be found in the internet at http://www.who.int/csr/sars/guidelines/en.

Positive test results: A positive SARS-CoV test indicates that the patient is infected with SARS-CoV even if SARS-related symptoms are not presented.

Negative test results: A negative SARS-CoV test does not exclude that the patient has SARS. The reasons for a negative test result despite SARS pathology may be:

- At the time of sample collection the virus was not present in the sample material (currently, it is not clear at which stage of the SARS-CoV pathogenesis the virus can be detected in a particular type of clinical specimen).
- The patient shows SARS-related symptoms that are caused by another infectious agent.

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* SARS RG RT-PCR Kit constitutes a ready-to-use system for the detection of SARS-CoV RNA using polymerase chain reaction (PCR) in the *artus 3000* or *Rotor-Gene 3000*. The *SARS-CoV RG/TM Master* contains reagents and enzymes for the reverse transcription and specific amplification of a 92 bp region of the SARS-CoV genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling A.FAM of the *artus 3000* or *Rotor-Gene 3000*. In addition, the *artus* SARS RG RT-PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an *Internal Control (IC)* in fluorescence channel Cycling A.JOE. The detection limit of the analytical SARS-CoV RT PCR (see 11.1 Analytical Sensitivity) is not reduced. External positive controls (*SARS-CoV LC/RG/TM*

QS 1 – 4) are supplied which allow the determination of the pathogen load. For further information, please refer to section 8.4 Quantitation.

8. Protocol

8.1 Pre-analytics: Specimen Collection, Storage and Transport

Precaution: All samples have to be treated as potentially infectious material.

Attention: Current studies refer to sputum as the most suitable sample material. Therefore, we recommend the use of this material with the *artus* SARS RG RT-PCR Kit.

The internal validation of the *artus* SARS RG RT-PCR Kit has been performed using serum samples. Other sample materials such as bronchoalveolar lavage (BAL), nasopharyngeal wash, swabs, lung tissue and sputum are not completely validated yet. Please use only recommended nucleic acid isolation kits (see 8.2 RNA Isolation) for sample preparation.

Using certain sample materials, particular instructions regarding collection, transport and storage have to be strictly observed.

Attention: Please consider the official recommendations of the WHO at the following webpage: http://www.who.int/csr/sars/guidelines/en.

8.1.1 Specimen Collection

For the collection of swabs, please use following materials:

Use only Dacron®- or rayon-tipped plastic swabs. Do not use aluminium or wooden swabs.

8.1.2 Sample Storage

The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer period of time.

Samples must be stored at 2 – 8°C. (If swab specimens are sent to a diagnostic laboratory, the samples have to be transported as soon as possible after collection and according to the instructions of the laboratory for the transport of SARS-CoV.)

If swab specimens are not processed directly after their receipt in the laboratory, they have to be stored at 2 – 8°C and have to be processed within one day. If they are not tested within one day after collection, the samples have to be stored at –20°C or colder and tested within up to 30 days after collection.

8.1.3 Sample Transport

Swab specimens must be transported cooled.

Swab specimens should be shipped to a laboratory as soon as possible after collection, following the laboratory instructions for transports under cooling. The samples should be transported following also the local and national instructions for the transport of pathogen material.*

8.2 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 kits are recommended:

Sample Material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer	Carrier RNA
Sputum, serum, BAL, nasopharyngeal wash, swabs	QIAamp Viral RNA Mini Kit (50)	52 904	QIAGEN	included
Lung tissue	RNeasy Mini Kit (50)	74 104	QIAGEN	not included

* International Air Transport Association. Dangerous Goods Regulations, 41st Edition, 2000. 704.

When using sputum as a sample material, please proceed as follows: For sample preparation, mix the sample in equal volumes with physiological salt solution (0.9 % NaCl) containing 1 % N-acetylcysteine (Sigma Cat. No.: A 8199) in a reaction tube, i.e. 300 µl sputum + 300 µl NaCl mix. After incubation of the mixture at room temperature for 30 min, use 140 µl of the lysate for the subsequent RNA purification with the QIAamp Viral RNA Mini Kit and continue with the viral RNA extraction protocol.

- 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 lyophilised carrier RNA prior to first use of the extraction kit in 310 µl of the elution buffer provided with the kit (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 the beginning of each extraction, a mixture of lysis buffer and carrier RNA (and *Internal Control*, where applicable, see 8.3 *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

  c) Please use the freshly prepared mixture of lysis buffer and carrier RNA 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* SARS RG RT-PCR Kit should not be used with **phenol**-based isolation methods.

Important: The *Internal Control* of the *artus* SARS RG RT-PCR Kit can be used directly in the isolation procedure (see 8.3 *Internal Control*).

8.3 *Internal Control*

An *Internal Control* (*SARS-CoV LC/RG/TM 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 QIAamp 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. The *Internal Control* and carrier RNA (see 8.2 RNA Isolation) should be added only

- to the mixture of lysis buffer and sample material or
- directly to the lysis buffer.

The *Internal Control* must not be added to the sample material directly. If added to the lysis buffer please note that the mixture of *Internal Control* and lysis buffer/carrier RNA 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* and the carrier RNA 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 1 µl of the *Internal Control* per reaction directly to 15 µl *SARS-CoV RG/TM Master*. For each PCR reaction use 15 µl of the Master Mix produced as described above* and add 10 µl of the purified sample. If you are preparing a PCR run for several samples please increase the volume of the *SARS-CoV RG/TM Master* and the *Internal Control* according to the number of samples (see 8.5 Preparing the PCR).

8.4 Quantitation

The enclosed *Quantitation Standards* (*SARS-CoV LC/RG/TM QS 1 – 4*) were calibrated against the standards provided by the Robert-Koch-Institute, Berlin, Germany. They are treated as previously purified samples and the same volume is used (10 µl). To generate a standard curve on the *artus 3000* or *Rotor-Gene 3000*, all four *Quantitation Standards* should be used and defined in the menu window *Edit Samples* as standards with the specified concentrations (see *artus 3000 Software Manual* or *Rotor-Gene Manual*, Version 4.6). 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 *artus 3000 Software Manual* or *Rotor-Gene Manual*, Version 4.6).

* 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.

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).

Important: A guideline for the quantitative analysis of *artus* systems on the *artus 3000* or *Rotor-Gene 3000* is provided at **www.qiagen.com/Products/ByLabFocus/MDX (Technical Note for quantitation on the *artus 3000* or *Rotor-Gene 3000*).**

8.5 Preparing the PCR

Make sure that the Cooling Block (accessory of the *artus 3000* or *Rotor-Gene 3000*) is pre-cooled to +4°C. Place the desired number of PCR tubes into 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* (*SARS-CoV LC/RG/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 8.3 *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	*SARS-CoV RG/TM Master*	15 µl	180 µl
	SARS-CoV LC/RG/TM IC	0 µl	0 µl
	Total Volume	15 µl	180 µl
2. Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample	10 µl	10 µl each
	Total Volume	25 µl	25 µl each

If you want to use the *Internal Control* exclusively to check for PCR inhibition, it must be added directly to the *SARS-CoV RG/TM 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	*SARS-CoV RG/TM Master*	15 µl	180 µl
	SARS-CoV LC/RG/TM IC	1 µl	12 µl
	Total Volume	16 µl*	192 µl*
2. Preparation of PCR assay	Master Mix	15 µl*	15 µl each*
	Sample	10 µl	10 µl each
	Total Volume	25 µl	25 µl each

* 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.

Pipette 15 µl of the Master Mix into each PCR tube. Then add 10 µl of the eluted sample RNA to each tube and mix well by pipetting up and down several times. Correspondingly, 10 µl of at least one of the *Quantitation Standards* (*SARS-CoV LC/RG/TM QS 1 – 4*) must be used as a positive control and 10 µl of water (*Water, PCR grade*) as a negative control. Close the PCR tubes. Please take care that the *Locking Ring* (accessory of the *artus 3000* or *Rotor-Gene 3000*) is placed on top of the rotor to prevent accidental opening of the tubes during the run.

Addition of the *Internal Control* to the Purification Procedure

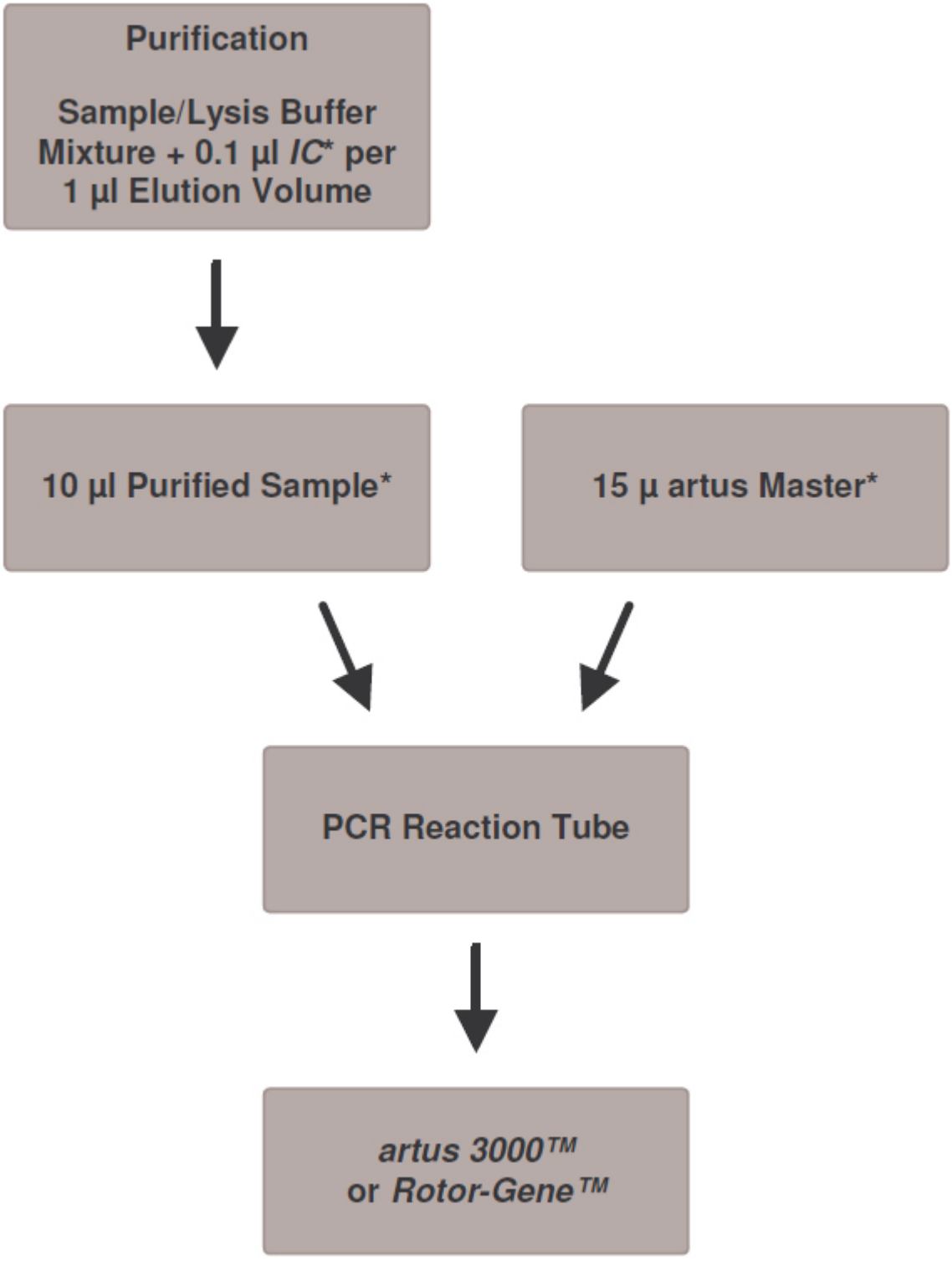

Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

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

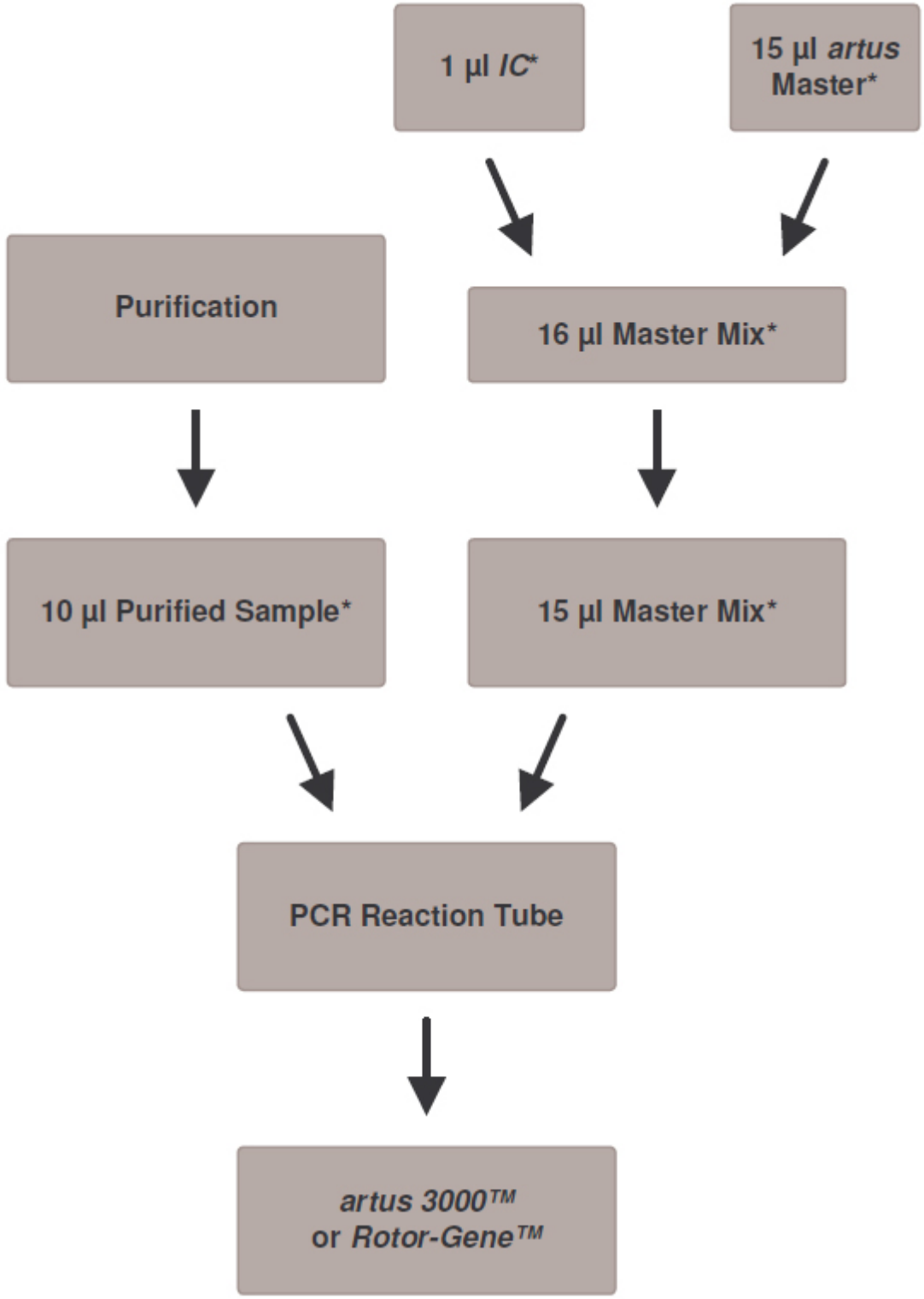

Fig. 2: Schematic workflow for the control of PCR inhibition.

* Please make sure that the solutions

8.6 Programming of the *artus 3000* or *Rotor-Gene 3000*

For the detection of SARS-CoV RNA, create a temperature profile on your *artus 3000* or *Rotor-Gene 3000* according to the following six steps (see Fig. 3 – Fig. 8).

A. Setting of General Assay Parameters Fig. 3

B. Reverse Transcription of the RNA Fig. 4

C. Initial Activation of the Hot Start Enzyme Fig. 5

D. Amplification of the cDNA Fig. 6

E. Adjustment of the Fluorescence Channel Sensitivity Fig. 7

F. Starting of the *artus 3000* or *Rotor-Gene 3000* Run Fig. 8

All specifications refer to the *artus 3000* software version 5.0.69 or *Rotor-Gene* software version 4.6.94. Please find further information on programming the *artus 3000* or *Rotor-Gene 3000* in the *artus 3000 Software Manual* or *Rotor-Gene Manual*, Version 4.6. In the illustrations these settings are framed in bold black.

First, enter the PCR reaction volume in the menu window *New Experiment Wizard* (see Fig. 3).

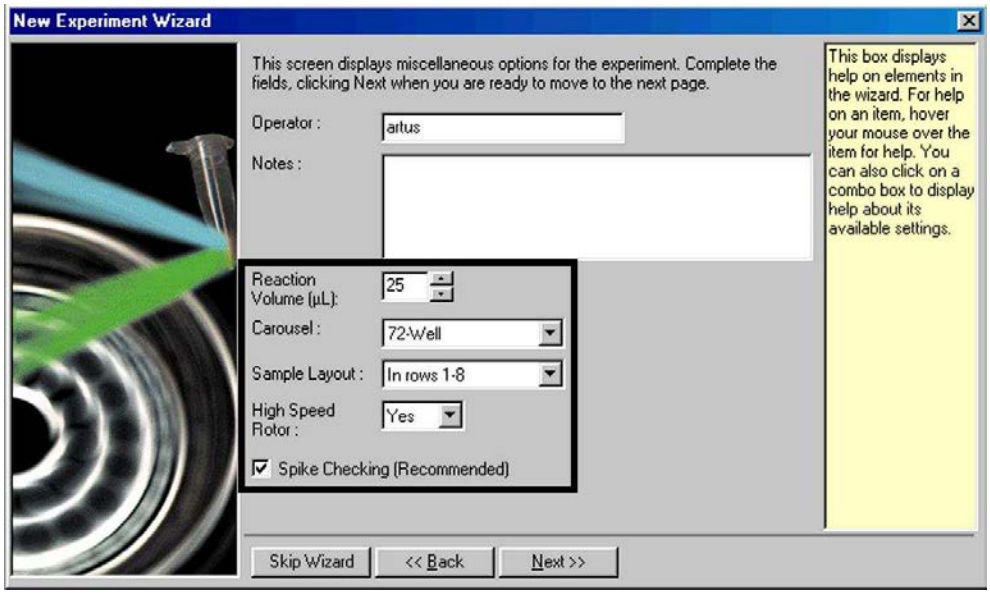

Fig. 3: Setting of General Assay Parameters.

Programming the temperature profile is done by activating the button *Edit* in the next *New Experiment Wizard* menu window (see Fig. 4, 5 and 6).

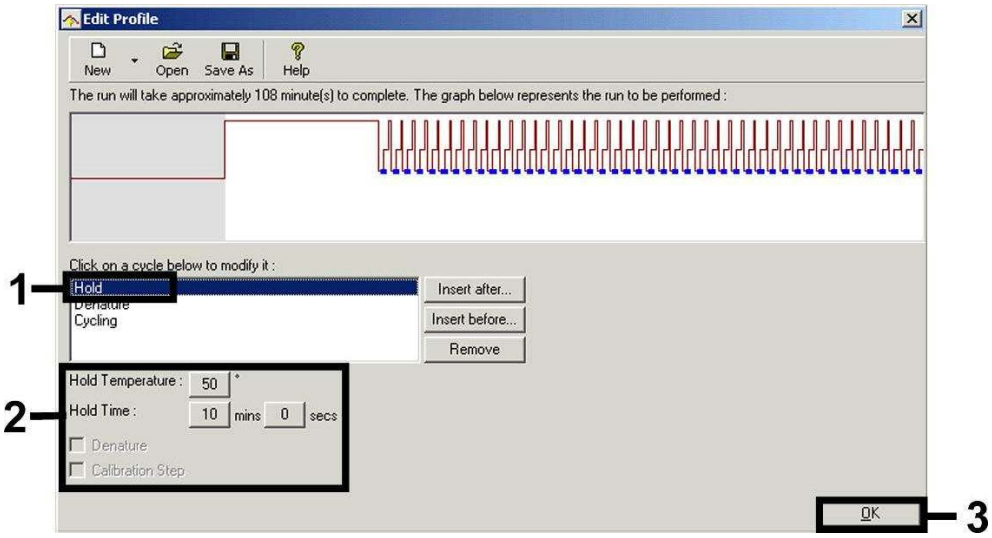

Fig. 4: Reverse Transcription of the RNA.

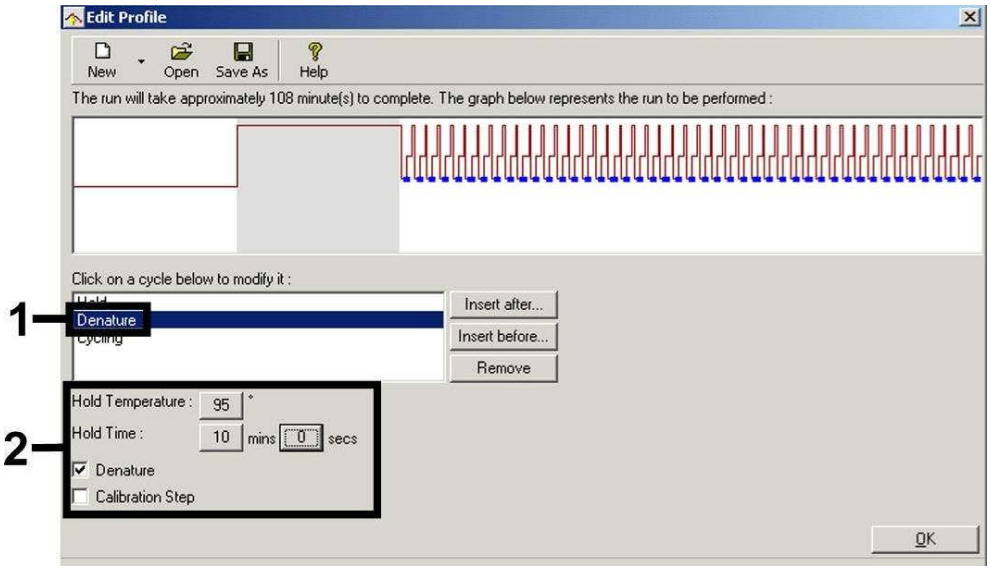

Fig. 5: Initial Activation of the Hot Start Enzyme.

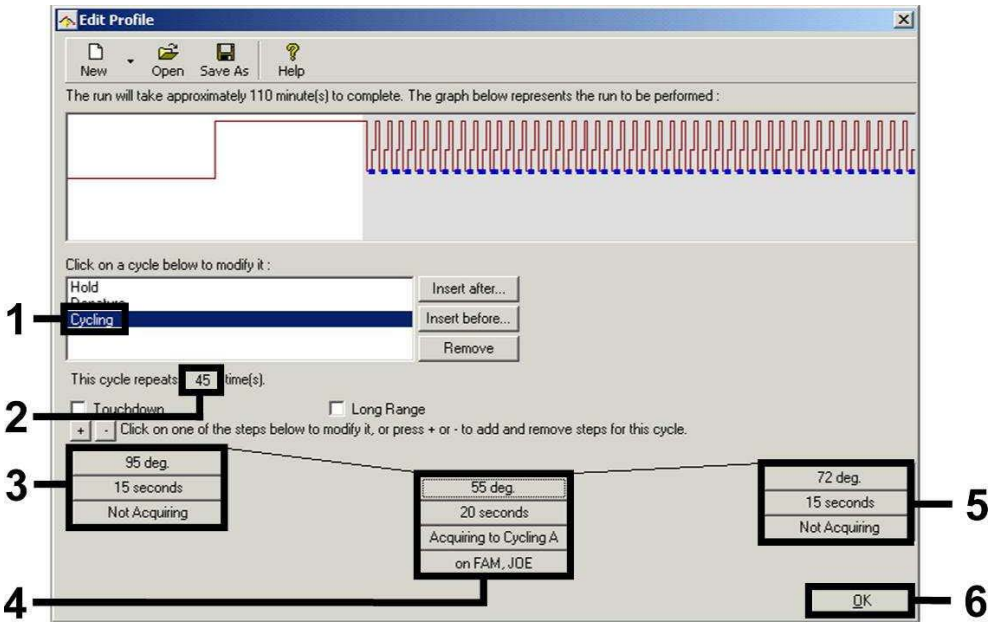

Fig. 6: Amplification of the cDNA.

The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. This adjustment is done in the menu window *Auto Gain Calibration Setup* (activation in menu window *New Experiment Wizard* under *Calibrate*). Please set the calibration temperature to the annealing temperature of the amplification programme (see Fig. 7).

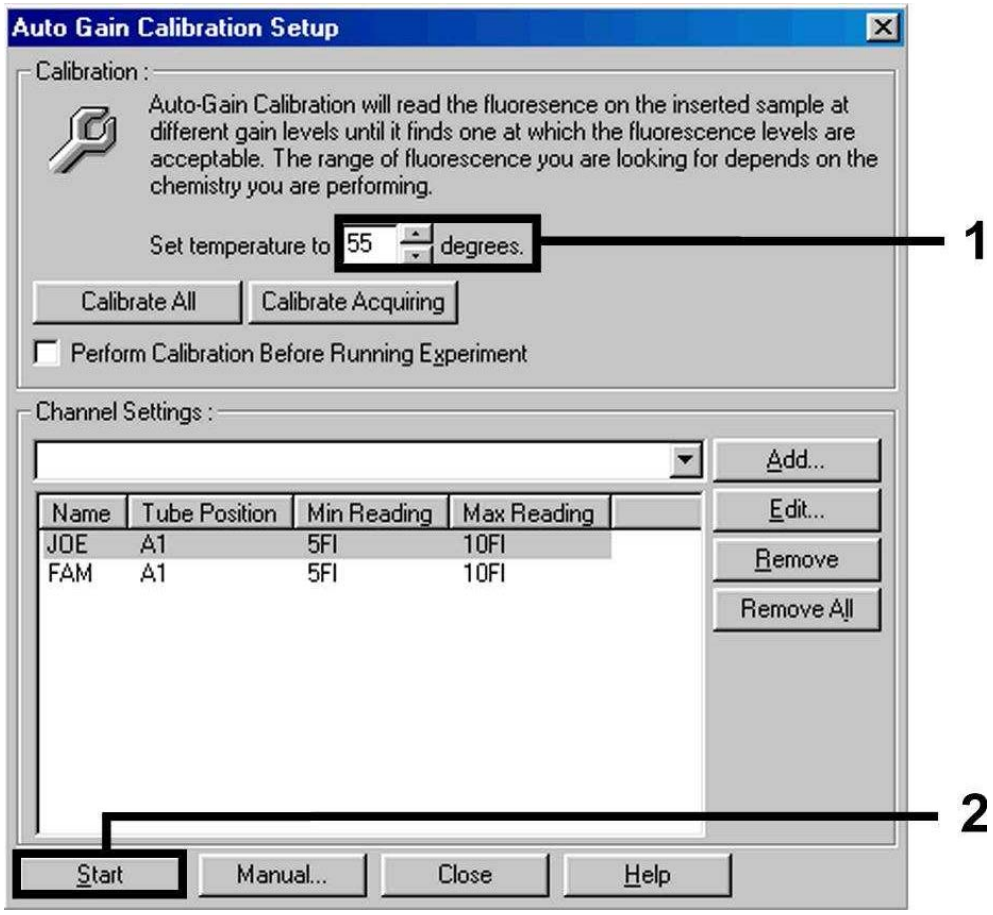

Fig. 7: Adjustment of the Fluorescence Channel Sensitivity.

The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (see Fig. 8).

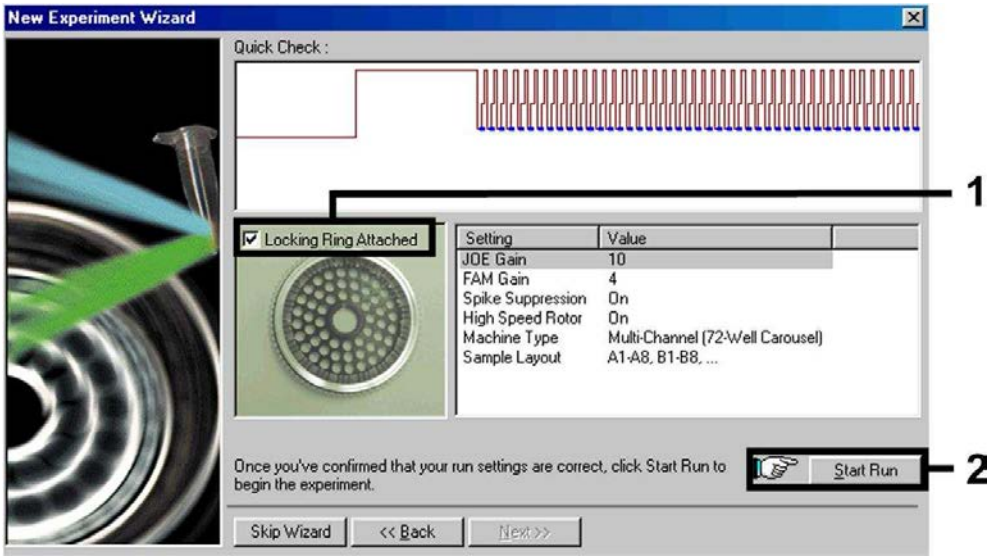

Fig. 8: Starting of the *artus 3000* or *Rotor-Gene 3000* Run.

9. Data Analysis

Data analysis is performed with the *artus 3000* or *Rotor-Gene* software according to the manufacturer's instructions (*artus 3000 Software Manual* or *Rotor-Gene Manual*, Version 4.6).

The following results are possible:

1. A signal is detected in fluorescence channel Cycling A.FAM.

   The result of the analysis is positive: The sample contains SARS-CoV RNA.

   In this case, the detection of a signal in the Cycling A.JOE channel is dispensable, since high initial concentrations of SARS-CoV RNA (positive signal in the Cycling A.FAM channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the Cycling A.JOE channel (competition).

2. In fluorescence channel Cycling A.FAM no signal is detected. At the same time, a signal from the *Internal Control* appears in the Cycling A.JOE channel.

   In the sample no SARS-CoV RNA is detectable. It can be considered negative.

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

3. No signal is detected in the Cycling A.FAM or in the Cycling A.JOE 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. 9 and Fig. 10.

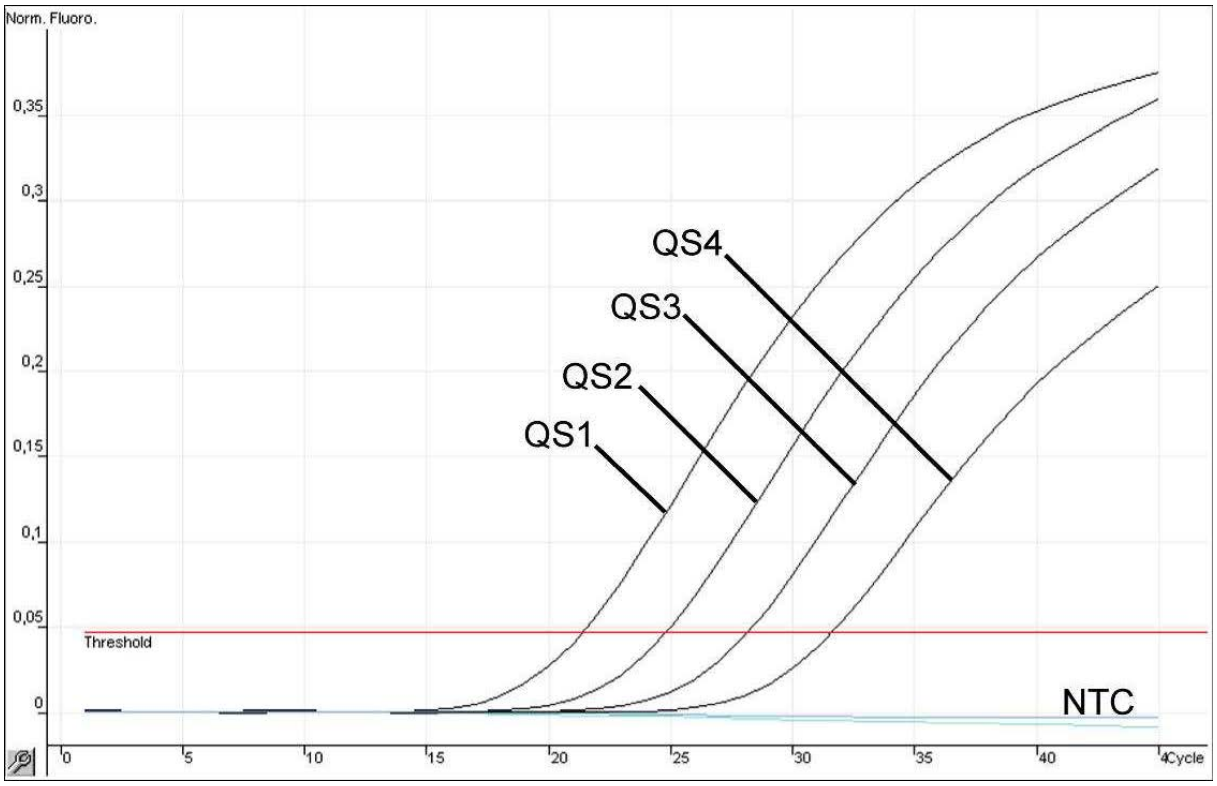

Fig. 9: Detection of the *Quantitation Standards* (*SARS-CoV LC/RG/TM QS 1 – 4*) in fluorescence channel Cycling A.FAM. NTC: non-template control (negative control).

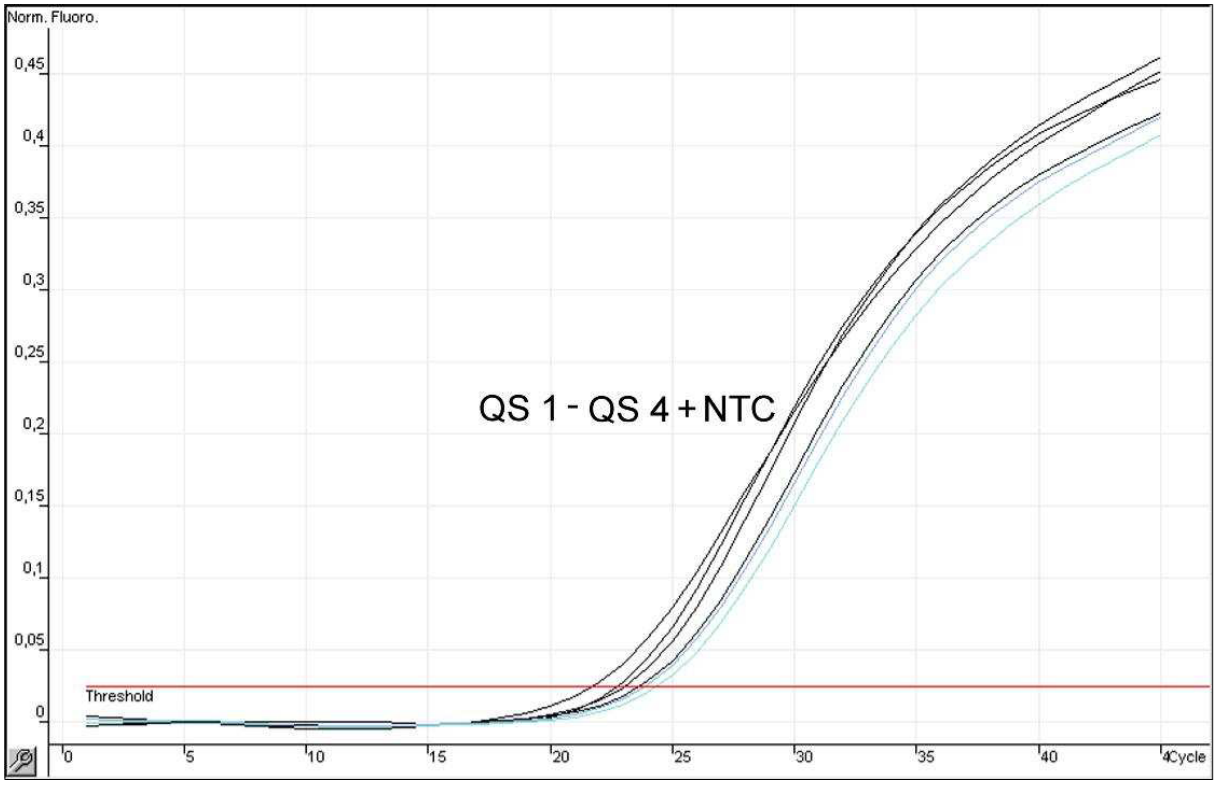

Fig. 10: Detection of the *Internal Control (IC)* in fluorescence channel Cycling A.JOE with simultaneous amplification of the *Quantitation Standards* (*SARS-CoV LC/RG/TM QS 1 – 4*). NTC: non-template control (negative control).

10. Troubleshooting

No signal with positive controls (*SARS-CoV LC/RG/TM QS 1 – 4*) in fluorescence channel Cycling A.FAM:

- The selected fluorescence channel for PCR data analysis does not comply with the protocol.

  ➔ For data analysis select the fluorescence channel A.FAM for the analytical SARS-CoV RT-PCR and the fluorescence channel A.JOE for the *Internal Control* RT-PCR.

- Incorrect programming of the temperature profile of the *artus 3000* or *Rotor-Gene 3000*.

  ➔ Compare the temperature profile with the protocol (see 8.6 Programming of the *artus 3000* or *Rotor-Gene 3000*).

- Incorrect configuration of the PCR reaction.

  ➔ Check your work steps by means of the pipetting scheme (see 8.5 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 SARS RG 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 fluorescence channel Cycling A.JOE and simultaneous absence of a signal in channel Cycling A.FAM:

- 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 8.2 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 8.2 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 8.2 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* SARS RG 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 fluorescence channel Cycling A.FAM of the analytical RT-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.

11. Specifications

11.1 Analytical Sensitivity

In order to determine the analytical sensitivity of the *artus* SARS RG RT-PCR Kit, a standard dilution series has been set up from 10 to nominal 0.003 of in vitro transcribed RNA copies per µl of the SARS-CoV amplicon and analysed with the *artus* SARS RG 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. 11. The analytical detection limit of the *artus* SARS RG RT-PCR Kit is consistently 0.5 copies/µl ($p = 0.05$). This means that there is a 95 % probability that 0.5 copies/µl will be detected.

Probit analysis: SARS Coronavirus (*artus 3000/Rotor-Gene 3000*)

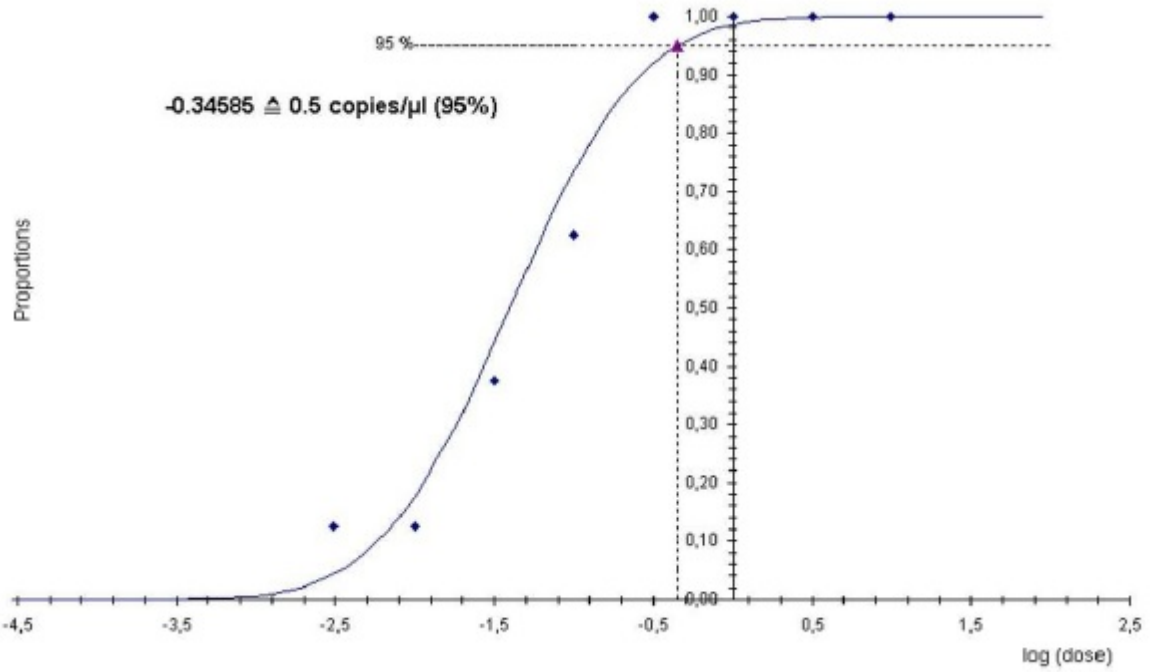

Fig. 11: Analytical sensitivity of the *artus* SARS RG RT-PCR Kit

11.2 Specificity

The specificity of the *artus* SARS RG 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 in gene banks published sequences by sequence comparison analysis. The detectability of all relevant sub-/genotypes has thus been ensured.

Moreover, the specificity was validated with 30 different SARS-CoV negative serum samples. These did not generate any signals with the SARS-CoV specific primers and probes, which are included in the *SARS-CoV RG/TM Master*.

To determine the specificity of the *artus* SARS RG 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	SARS-CoV (Cycling A.FAM)	*Internal Control* (Cycling A.JOE)
HCoV OC 43 ATCC (Human coronavirus OC 43)	–	+
HCoV 229 E ATCC (Human coronavirus 229 E)	–	+
SB 1 + 4 HCoV (Human coronavirus SB 1 + 4)	–	+
SB 164 HCoV (Human coronavirus SB 164)	–	+
IBV Beaudelle (Avian infectious bronchitis virus Beaudelle)	–	+
BCV 212 (Bovine CoV 212)	–	+
TGEV Perdue (Porcine transmissible gastroenteritis virus Perdue)	–	+
TGEV Pur 46 C 188 (Porcine transmissible gastroenteritis virus Pur)	–	+

11.3 Precision

The precision data of the *artus* SARS RG 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* SARS RG RT-PCR Kit have been collected using the *Quantitation Standard* of the lowest concentration (QS 4; 10 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). Based on these results, the overall statistical spread of any given

sample with the mentioned concentration is 1.66 %, for the detection of the *Internal Control* 1.28 %. 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: *SARS-CoV LC/RG/TM QS 4*	0.15	0.02	0.48
Intra-assay variability: *Internal Control*	0.40	0.15	1.67
Inter-assay variability: *SARS-CoV LC/RG/TM QS 4*	0.23	0.05	0.75
Inter-assay variability: *Internal Control*	1.13	1.28	4.53
Inter-batch variability: *SARS-CoV LC/RG/TM QS 4*	0.52	0.25	1.69
Inter-batch variability: *Internal Control*	0.94	0.63	3.62
Total variance: *SARS-CoV LC/RG/TM QS 4*	0.51	0.26	1.66
Total variance: *Internal Control*	1.13	1.28	1.28

11.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* SARS RG RT-PCR Kit. 30 SARS-CoV negative samples of serum were spiked with 1.5 copies/µl elution volume of SARS-CoV control RNA (threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp Viral RNA Mini Kit (see 8.2 RNA Isolation) these samples were analysed with the *artus* SARS RG RT-PCR Kit. For all SARS-CoV samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 30 SARS-CoV negative serum samples. The total failure rate was 0 %. Inhibitions were not observed. Thus, the robustness of the *artus* SARS RG RT-PCR Kit is ≥ 99 %.

11.5 Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* SARS RG RT-PCR Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programmes.

11.6 Diagnostic Evaluation

Currently, the *artus* SARS RG 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.

13. Safety information

For safety information of the *artus* SARS RG 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.

14. Quality control

In accordance with QIAGEN's ISO 9001 and ISO 13485-certified Quality Management System, each lot of *artus* SARS RG RT-PCR Kit has been 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

Catalogue number

Material number

Handbook

In vitro diagnostic medical device

Ethanol

Global Trade Item Number

<N>
Contains sufficient for <N> tests


Temperature limitation

QS *Quantitation Standard*

IC *Internal Control*

Notes

Notes

Notes

artus SARS RG RT-PCR Kit

Trademarks and Disclaimers

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The *artus* SARS RG RT-PCR Kit is a CE-marked diagnostic kit according to the European In Vitro Diagnostic Directive 98/79/EC. Not available in all countries.

The QIAamp Kits are intended for general laboratory use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Purchase of *artus* PCR Kits is accompanied by a limited license to use them in the polymerase chain reaction (PCR) process for human and veterinary in vitro diagnostics in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler. The PCR process is covered by the foreign counterparts of U.S. Patents Nos. 5,219,727 and 5,322,770 and 5,210,015 and 5,176,995 and 6,040,166 and 6,197,563 and 5,994,056 and 6,171,785 and 5,487,972 and 5,804,375 and 5,407,800 and 5,310,652 and 5,994,056 owned by F. Hoffmann-La Roche Ltd.

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