

# artus<sup>®</sup> M. tuberculosis RG PCR Kit

## Handbook



24 (catalog no. 4555203)



96 (catalog no. 4555205)

For research use only. Not for use in diagnostic procedures.

For use with the *artus 3000*<sup>™</sup> and the *Rotor-Gene*<sup>™</sup> 3000

June 2007 – Version 1



4555203, 4555205



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artus M. tuberculosis RG PCR Kit

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The artus M. tuberculosis RG PCR Kit is for research use only. Not for use in diagnostic procedures.

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.

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## artus<sup>®</sup> M. tuberculosis RG PCR Kit

For use with the *artus 3000*<sup>™</sup> or the *Rotor-Gene*<sup>™</sup> 3000<sup>\*</sup>.

**For research use only. Not for use in diagnostic procedures.**

### 1. Contents

	Labelling and contents	Art. No. 4555203 24 reactions	Art. No. 4555205 96 reactions
Blue	<i>M. tuberculosis</i> RG Master	2 x 12 rxns	8 x 12 rxns
Yellow	<i>M. tuberculosis</i> RG Mg-Sol <sup>‡</sup>	1 x 400 µl	1 x 400 µl
Red	<i>M. tuberculosis</i> RG/TM QS 1 <sup>‡</sup> 3 x 10 <sup>4</sup> cop/µl	1 x 200 µl	1 x 200 µl
Red	<i>M. tuberculosis</i> RG/TM QS 2 <sup>‡</sup> 3 x 10 <sup>3</sup> cop/µl	1 x 200 µl	1 x 200 µl
Red	<i>M. tuberculosis</i> RG/TM QS 3 <sup>‡</sup> 3 x 10 <sup>2</sup> cop/µl	1 x 200 µl	1 x 200 µl
Red	<i>M. tuberculosis</i> RG/TM QS 4 <sup>‡</sup> 3 x 10 <sup>1</sup> cop/µl	1 x 200 µl	1 x 200 µl
Green	<i>M. tuberculosis</i> RG IC <sup>‡</sup>	1 x 1,000 µl	2 x 1,000 µl
White	Water (PCR grade)	1 x 1,000 µl	1 x 1,000 µl

<sup>‡</sup> QS = Quantitation Standard  
IC = Internal Control  
Mg-Sol = Magnesium Solution

### 2. Storage

The components of the *artus* M. tuberculosis RG PCR Kit should be stored at -20°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.

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<sup>\*</sup> The *artus* M. tuberculosis RG PCR Kit can be used with the *Rotor-Gene*<sup>™</sup> 2000 as well.

### 3. Additionally Required Materials and Devices

- Disposable powder-free gloves
- DNA isolation kit (see **8.1 DNA Isolation**)
- Lysozyme mix (see **8.1 DNA Isolation**)
- Pipettes (adjustable)
- 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

Tuberculosis (TB) is still one of the most important infectious diseases worldwide. Some two billion people, one-third of the world's population, are infected with *Mycobacterium tuberculosis*, the causative agent of tuberculosis. The incidence of tuberculosis worldwide is about eight million and about three million people die each year. Even though particularly the Third World countries are affected. Tuberculosis is a reemerging disease also in industrialized nations, mainly due to the immigration of infected people and the development of drug resistant TB. Minorities like homeless, drug users and immunocompromised persons are affected disproportionately.

Tuberculosis is a chronic, cyclic disease, mainly affecting the lung and the associated lymph nodes. However, depending on the immune status of the patient, the *M. tuberculosis* bacteria can also colonize other organs. TB is transmitted from person to person via aerosols. Only people with active disease are contagious. Especially in immunosuppressed people *M. tuberculosis* bacteria can be reactivated (recrudescent), even years after the initial infection.

## 6. 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 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* *M. tuberculosis* RG PCR Kit constitutes a ready-to-use system for the detection of DNA of all members of the *M. tuberculosis* complex (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis* BCG, *M. microti*, *M. pinnipedii*) using polymerase chain reaction (PCR) in the *artus 3000*<sup>™</sup> or *Rotor-Gene*<sup>™</sup> 3000. The *M. tuberculosis* RG Master contains reagents and enzymes for the specific amplification of a 159 bp region of the mycobacterial genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling A.FAM of the *artus 3000*<sup>™</sup> or *Rotor-Gene*<sup>™</sup> 3000. In addition, the *artus* *M. tuberculosis* RG 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 *M. tuberculosis* complex PCR (see **11.1 Analytical Sensitivity**) is not reduced. External positive controls (*M. tuberculosis* RG/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 DNA Isolation

Before the DNA isolation, large sample volumes or strongly acidic samples must first be concentrated or neutralized, respectively. For the analysis of sputum, we recommend a NALC-NaOH decontamination; stomach fluid should be neutralized with phosphate buffer. After a final centrifugation, the bacteria pellet can be used for the following DNA isolation.

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

Sample Material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer	Carrier RNA
Sputum, BAL, bronchial secretion, CSF, stomach fluid, peritoneal puncture	QIAamp DNA Mini Kit (50)	51 304	QIAGEN	not included

**Attention:** It is important to follow the instructions in **Protocol D** (Protocols for bacteria) described in the *QIAGEN Kit User Manual*. To ensure an effective and contamination-free lysis of the mycobacteria the following amendments (highlighted bold) of QIAamp DNA Mini Kit supplement Protocol D are highly recommended:

**Important:** All pipetting steps before the incubation at 95°C have to be performed in a class II safety cabinet, since the samples are potentially infectious.

- **Transfer between 250 µl and 500 µl of the NALC-NaOH-decontaminated sample into a 1.5 ml screw cap tube.**
- Centrifuge 10 minutes at **17,000 x g (13,000 rpm)** in a desktop centrifuge.
- **Carefully discard the supernatant by pipetting.**
- Add 180 µl lysozyme mix (20 mg/ml lysozyme; 20 mM Tris-HCl (pH 8.0); 2 mM EDTA; 1.2 % Triton®) and resuspend the pellet by pipetting up and down.
- Incubate for **at least one hour** at 37°C in a thermoblock.
- **Centrifuge briefly to remove drops from the inside of the lid.**
- Add 20 µl Proteinase K and 200 µl AL buffer **supplemented with carrier RNA** (2 µg RNA-Homopolymer Poly(A), Amersham Biosciences, per 200 µl AL buffer) **and optionally 10 µl Internal Control** (see 8.2 Internal Control).
- Mix well by vortexing.
- Incubate for 30 minutes at 56°C in a thermoblock.
- **Centrifuge briefly to remove drops from the inside of the lid.**
- Incubate 15 minutes at 95°C (**incubation time should not be exceeded as this may cause DNA degradation**).

**Important:** Please note that only after completion of the incubation at 95 °C the samples are no longer infectious.

- **Cool the sample down to room temperature.**
- Centrifuge briefly to remove drops from the inside of the lid.
- Follow the Tissue Protocol in the QIAGEN Kit User Manual starting with step 4 and **perform the final DNA elution using 100 µl AE buffer.**

**Important remarks for the prevention of cross-contaminations:**

**a) Regarding the lysis of the bacteria:**

- The use of screw cap tubes is absolutely essential.
- The screw cap tubes must always be locked tightly.
- After each incubation step centrifuge the tube briefly to remove drops from the inside of the lid.
- Do not touch the inside of the tube lid. If so, please change the potentially contaminated glove immediately.
- The use of a waterbath is generally not recommended.
- Make sure the samples cool down to room temperature after the 95°C heating step, since otherwise the risk of aerosol-mediated contamination after opening the tube is extremely high.

**b) Regarding the DNA isolation**

- Make sure that you do not wet the rim of a QIAamp spin column.
- Do not touch the lid of a QIAamp spin column on the inside. If so, change the potentially contaminated glove immediately.
- Do not use the same pipette tip for different samples, not even to apply the washing buffers AW1 and AW2 or the elution buffer AE. This avoids cross-contamination between samples and the contamination of a buffer.
- Use each 2 ml collection tube only once. If you run out of collection tubes you may also use 2 ml microcentrifuge tubes, the lids of which have to be removed before.

### General remarks:

- The use of **carrier RNA** is critical for the extraction efficiency and, consequently, for DNA/RNA yield. If the selected isolation kit does not contain carrier RNA, please note that the addition of carrier (RNA-Homopolymer Poly(A), Amersham Biosciences, Cat. No. 27-4110-01) is strongly recommended for the extraction of nucleic acids from cell free body fluids and material low in DNA/RNA content (e.g. CSF). Please proceed as follows in these cases:
  - a) Resuspend the lyophilised carrier RNA using the elution buffer (do not use lysis buffer) of the extraction kit (e.g. AE buffer of the QIAamp DNA Mini Kit) and prepare a dilution with a concentration of 1 µg/µl. Divide this carrier RNA solution in 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) Use 1 µg carrier RNA per 100 µl lysis buffer. For instance, if the extraction protocol suggests 200 µl lysis buffer, please add 2 µl carrier RNA (1 µg/µl) directly into the lysis buffer. 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
Lysis buffer	e.g. 200 µl	e.g. 2,400 µl
Carrier RNA (1 µg/µl)	2 µl	24 µl
<b>Total Volume</b>	<b>202 µl</b>	<b>2,424 µl</b>
<b>Volume per extraction</b>	<b>200 µl</b>	<b>each 200 µl</b>

- 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 M. tuberculosis* RG PCR Kit should not be used with **phenol-**based isolation methods.

**Important:** The *Internal Control* of the *artus M. tuberculosis* RG PCR Kit can be used directly in the isolation procedure (see **8.2 Internal Control**).

## 8.2 Internal Control

An *Internal Control* (*M. tuberculosis* RG IC) is supplied. This allows the user **both to control the DNA 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 DNA Mini Kit the DNA is eluted in 100 µl AE buffer. Hence, 10 µl of the *Internal Control* should be added initially. The quantity of *Internal Control* used depends **only** on the elution volume. The *Internal Control* and carrier RNA (see **8.1 DNA 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 0.5 µl of the *Internal Control* and 2 µl *M. tuberculosis* RG Mg-Sol per reaction directly to 13 µl *M. tuberculosis* RG Master. For each PCR reaction use 15 µl of the

Master Mix produced as described above<sup>\*</sup> and add 10 µl of the purified sample. If you are preparing a PCR run for several samples please increase the volume of the *M. tuberculosis* RG Master, the *M. tuberculosis* RG Mg-Sol and the *Internal Control* according to the number of samples (see **8.4 Preparing the PCR**).

### 8.3 Quantitation

The enclosed *Quantitation Standards (M. tuberculosis RG/TM QS 1 - 4)* 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). 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:

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

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).

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<sup>\*</sup> 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 *artus 3000™* or *Rotor-Gene™ 3000* is provided at [www.qiagen.com/Products/ByLabFocus/MDX](http://www.qiagen.com/Products/ByLabFocus/MDX) (Technical Note for quantitation on the *artus 3000™* or *Rotor-Gene™ 3000*).

## 8.4 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 (M. tuberculosis 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 quick vortexing) and centrifuged briefly.

If you want to use the *Internal Control* to check **not only possible PCR inhibition but also the DNA isolation procedure**, 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	<i>M. tuberculosis RG Master</i>	13 µl	156 µl
	<i>M. tuberculosis RG Mg-Sol</i>	2 µl	24 µl
	<i>M. tuberculosis RG IC</i>	0 µl	0 µl
	<b>Total Volume</b>	<b>15 µl</b>	<b>180 µl</b>
2. Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample	10 µl	10 µl each
	<b>Total Volume</b>	<b>25 µl</b>	<b>25 µl each</b>

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

	Number of samples	1	12
<b>1. Preparation of Master Mix</b>	<i>M. tuberculosis</i> RG Master	13 µl	156 µl
	<i>M. tuberculosis</i> RG Mg-Sol	2 µl	24 µl
	<i>M. tuberculosis</i> RG IC	0.5 µl	6 µl
	<b>Total Volume</b>	<b>15.5 µl*</b>	<b>186 µl*</b>
<b>2. Preparation of PCR assay</b>	Master Mix	15 µl*	15 µl each*
	Sample	10 µl	10 µl each
	<b>Total Volume</b>	<b>25 µl</b>	<b>25 µl each</b>

Pipette 15 µl of the Master Mix into each PCR tube. Then add 10 µl of the eluted sample DNA to each tube and mix well by pipetting up and down several times. Correspondingly, 10 µl of at least one of the *Quantitation Standards (M. tuberculosis* 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.

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\* 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

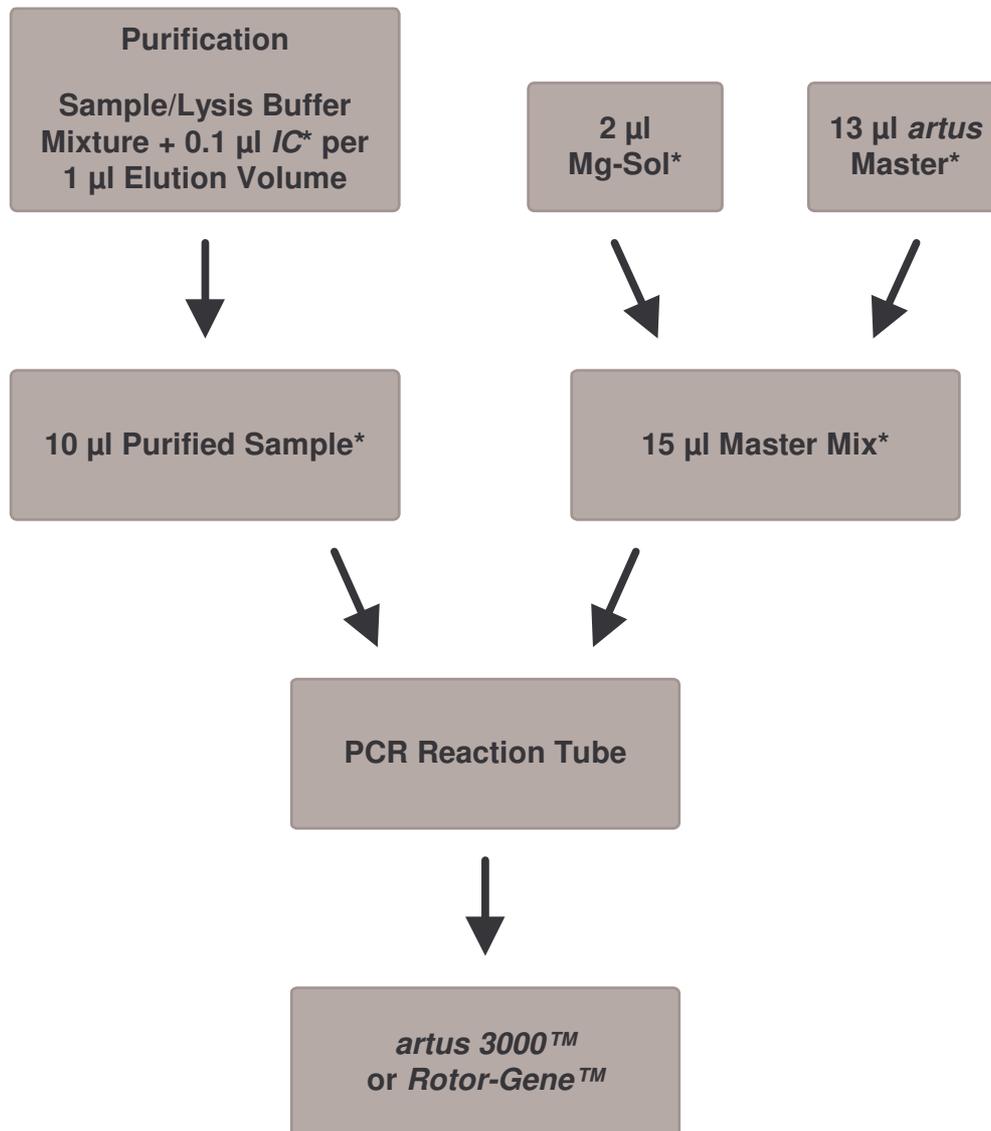


Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

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

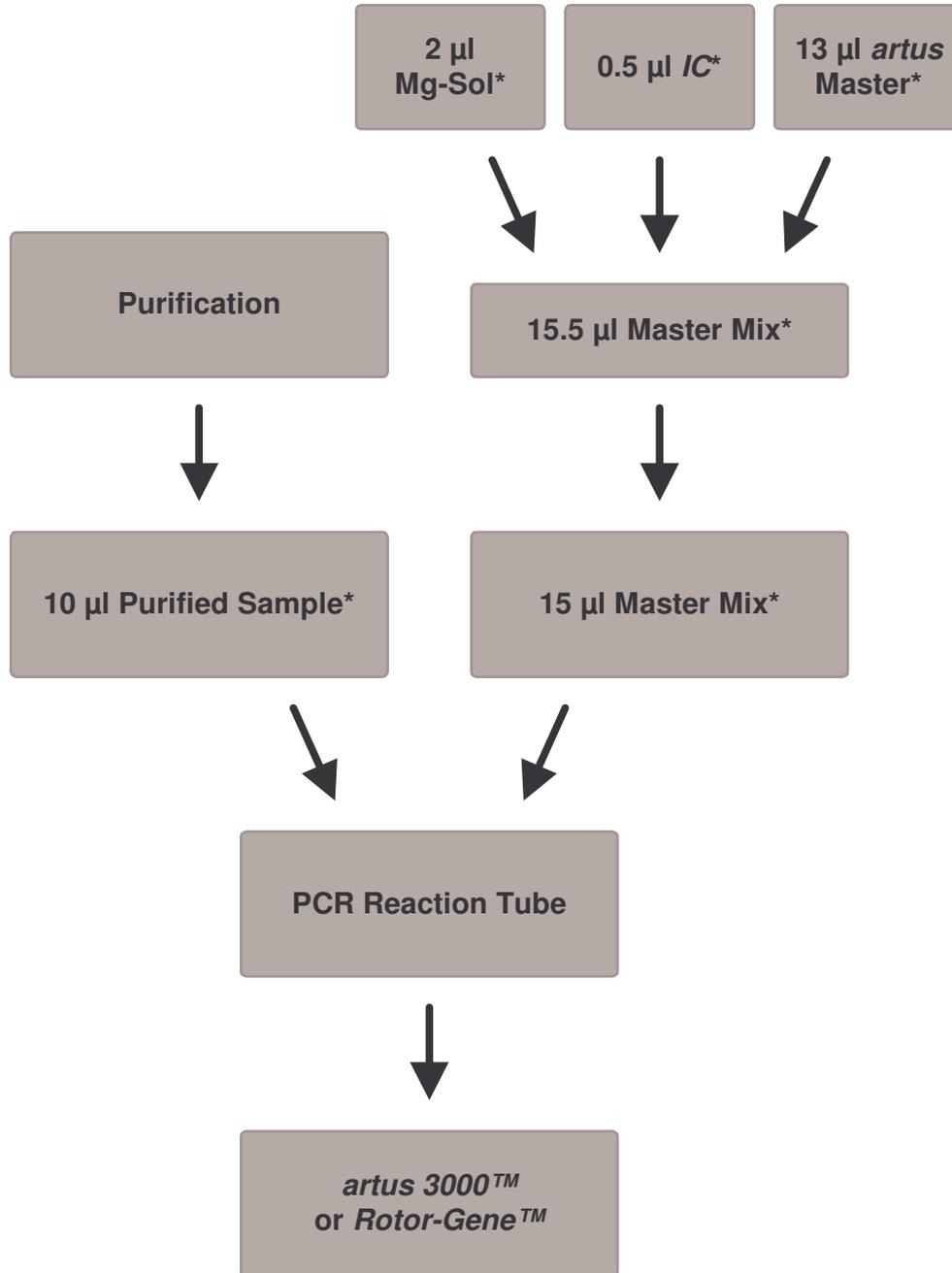


Fig. 2: Schematic workflow for the control of PCR inhibition.

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

## 8.5 Programming of the *artus 3000*<sup>TM</sup> or *Rotor-Gene*<sup>TM</sup> 3000

For the detection of DNA of all members of the *M. tuberculosis* complex, create a temperature profile on your *artus 3000*<sup>TM</sup> or *Rotor-Gene*<sup>TM</sup> 3000 according to the following five steps (see Fig. 3 - 7).

- A. Setting of General Assay Parameters Fig. 3
- B. Initial Activation of the Hot Start Enzyme Fig. 4
- C. Amplification of the DNA Fig. 5
- D. Adjustment of the Fluorescence Channel Sensitivity Fig. 6
- E. Starting of the *artus 3000*<sup>TM</sup> or *Rotor-Gene*<sup>TM</sup> 3000 Run Fig. 7

All specifications refer to the *artus 3000*<sup>TM</sup> software version 5.0.69 or *Rotor-Gene*<sup>TM</sup> software version 4.6.94. Please find further information on programming the *artus 3000*<sup>TM</sup> or *Rotor-Gene*<sup>TM</sup> 3000 in the *artus 3000*<sup>TM</sup> *Software Manual* or *Rotor-Gene*<sup>TM</sup> *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).

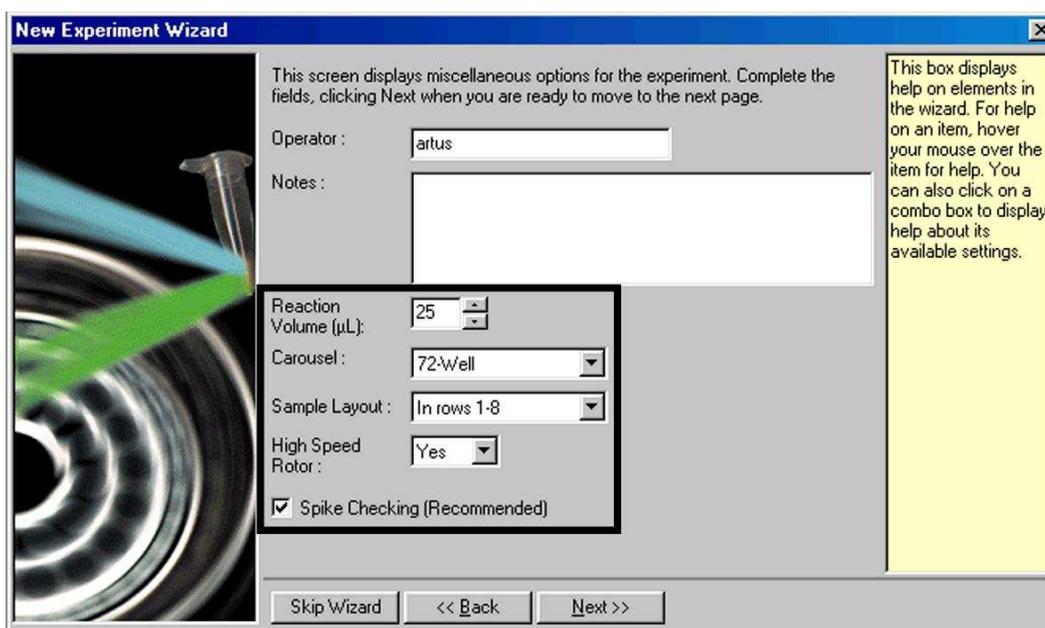


Fig. 3: Setting of General Assay Parameters.

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

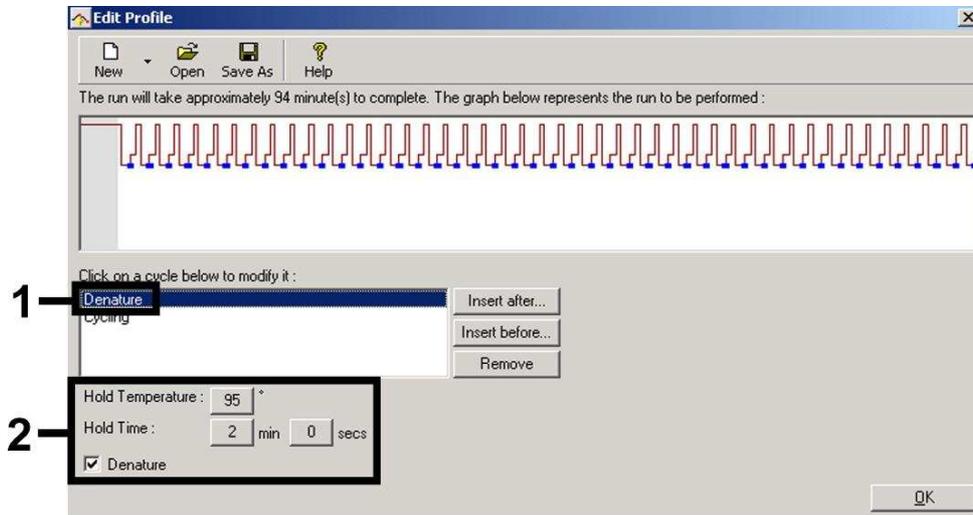


Fig. 4: Initial Activation of the Hot Start Enzyme.

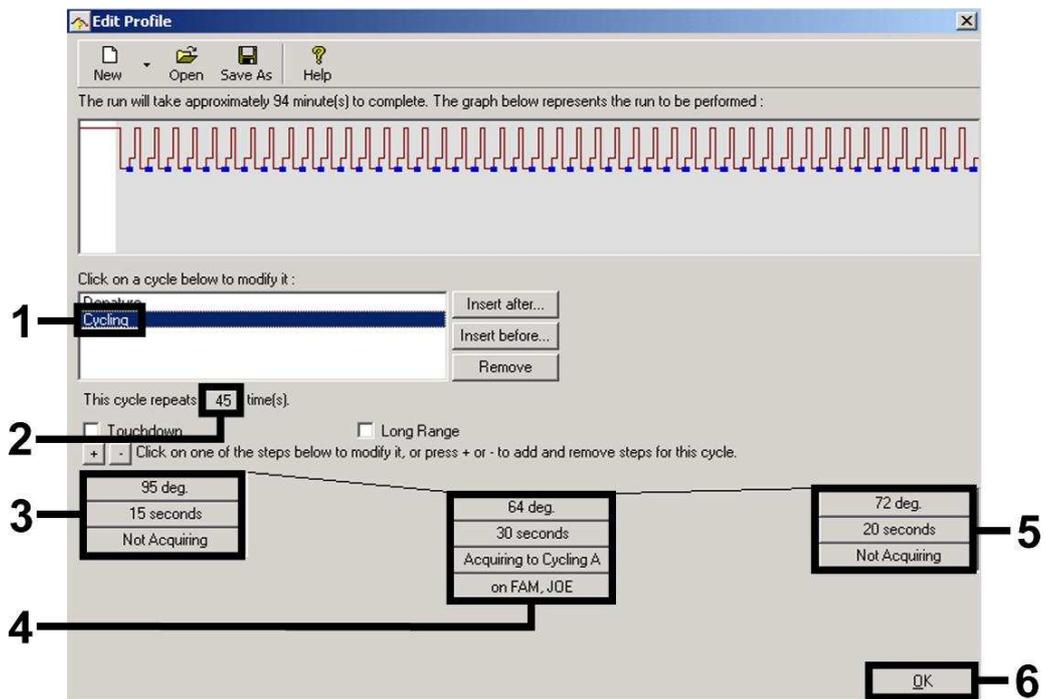


Fig. 5: Amplification of the DNA.

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. 6).

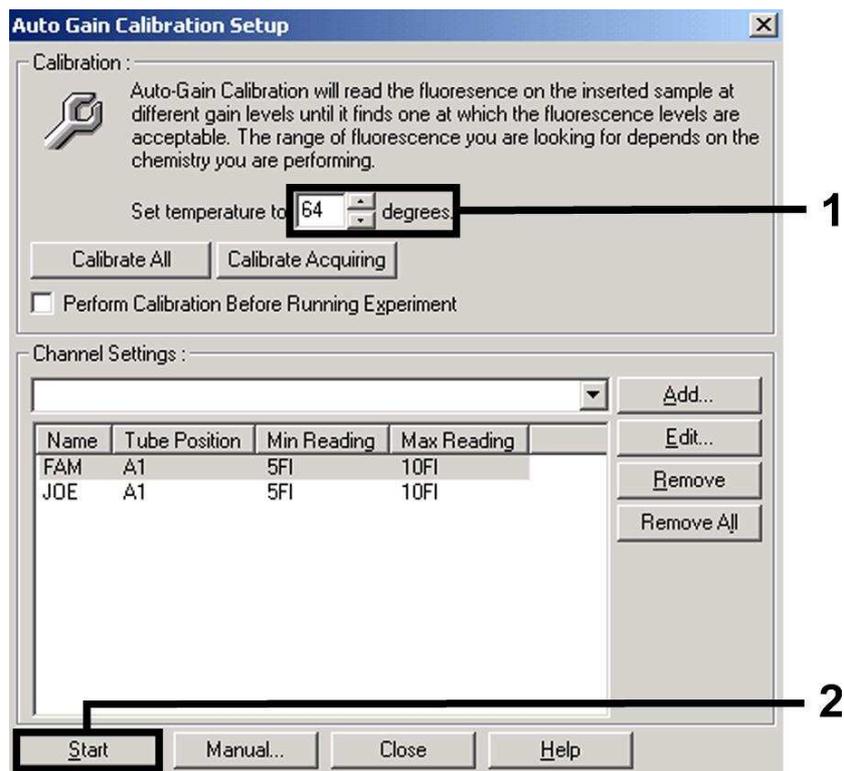


Fig. 6: Adjustment of the Fluorescence Channel Sensitivity.

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. 7).

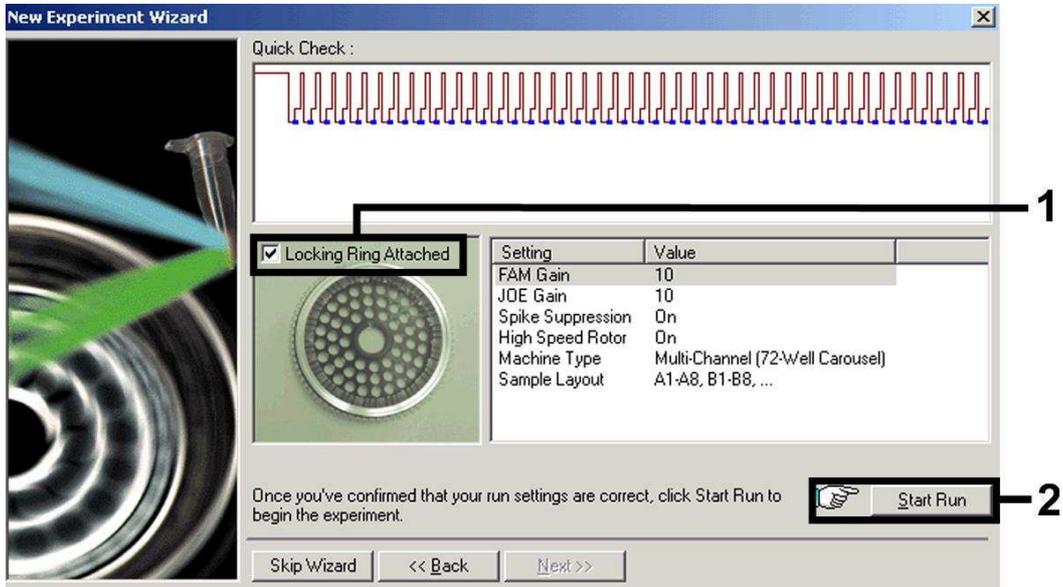


Fig. 7: Starting of the *artus 3000*<sup>TM</sup> or *Rotor-Gene*<sup>TM</sup> 3000 Run.

## 9. Data Analysis

Data analysis is performed with the *artus 3000*<sup>TM</sup> or *Rotor-Gene*<sup>TM</sup> software according to the manufacturer's instructions (*artus 3000*<sup>TM</sup> *Software Manual* or *Rotor-Gene*<sup>TM</sup> *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 DNA of one or more members of the *M. tuberculosis* complex.**

In this case, the detection of a signal in the Cycling A.JOE channel is dispensable, since high initial concentrations of *M. tuberculosis* complex DNA (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 DNA of members of the *M. tuberculosis* complex is detectable. It can be considered negative.**

In the case of a negative *M. tuberculosis* complex PCR the detected signal of the *Internal Control* rules out the possibility of PCR inhibition.

3. No signal is detected in the Cycling A.FAM or in the Cycling A.JOE channel.

**No result 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. 8 and Fig. 9.

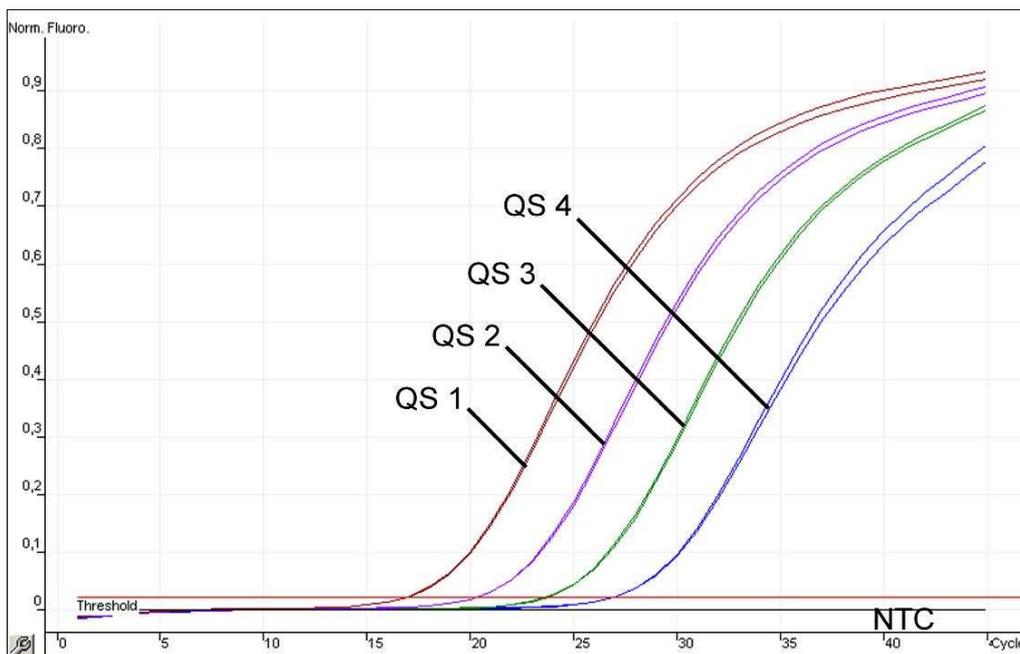


Fig. 8: Detection of the *Quantitation Standards* (*M. tuberculosis* RG/TM QS 1 - 4) in fluorescence channel Cycling A.FAM. NTC: non-template control (negative control).

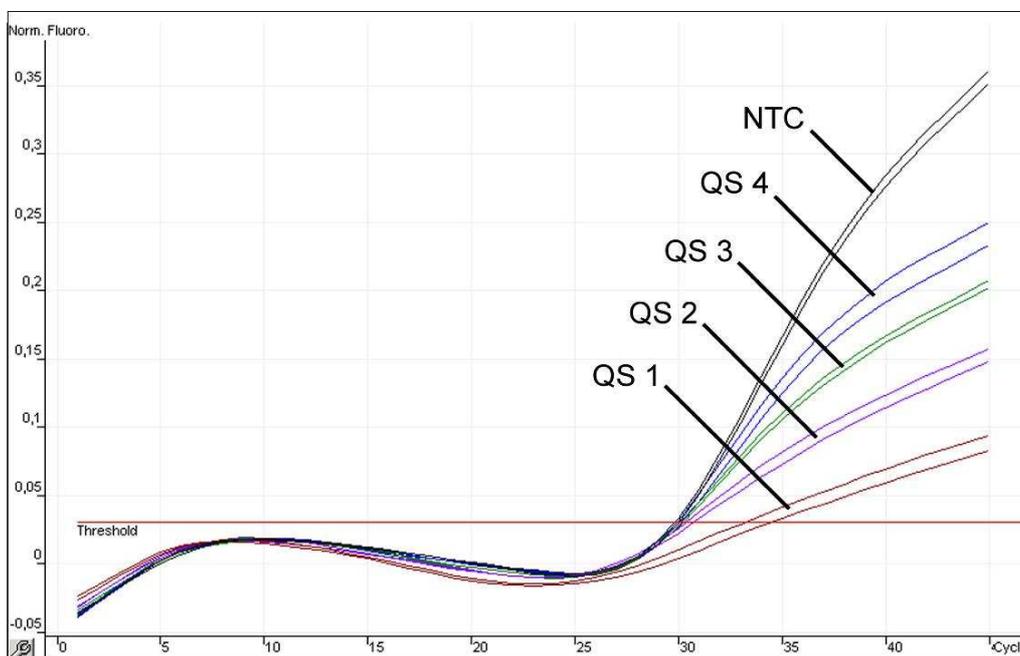


Fig. 9: Detection of the *Internal Control* (IC) in fluorescence channel Cycling A.JOE with simultaneous amplification of the *Quantitation Standards* (*M. tuberculosis* RG/TM QS 1 - 4). NTC: non-template control (negative control).

## 10. Troubleshooting

### No signal with positive controls (*M. tuberculosis* 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 *M. tuberculosis* complex PCR and the fluorescence channel A.JOE for the *Internal Control* PCR.
- Incorrect programming of the temperature profile of the *artus 3000™* or *Rotor-Gene™ 3000*.
  - Compare the temperature profile with the protocol (see **8.5 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 **8.4 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 **0**.
- Storage of the *artus M. tuberculosis* RG 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 for the specific *M. tuberculosis* complex PCR:

- 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 DNA Isolation**) and stick closely to the manufacturer's instructions.

- Make sure that during the DNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see **8.1 DNA Isolation**).
- DNA 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 DNA during the extraction. Make sure that you use a recommended isolation method (see **8.1 DNA 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 **0**.
- Storage of the *artus M. tuberculosis RG* 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 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* M. tuberculosis RG PCR Kit, a standard dilution series has been set up from 10 to nominal 0.05 *M. tuberculosis* copy equivalents<sup>\*</sup>/μl and analyzed with the *artus* M. tuberculosis RG 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. 10. The analytical detection limit of the *artus* M. tuberculosis RG PCR Kit is consistently 0.9 copies/μl (p = 0.05). This means that there is a 95 % probability that 0.9 copies/μl will be detected.

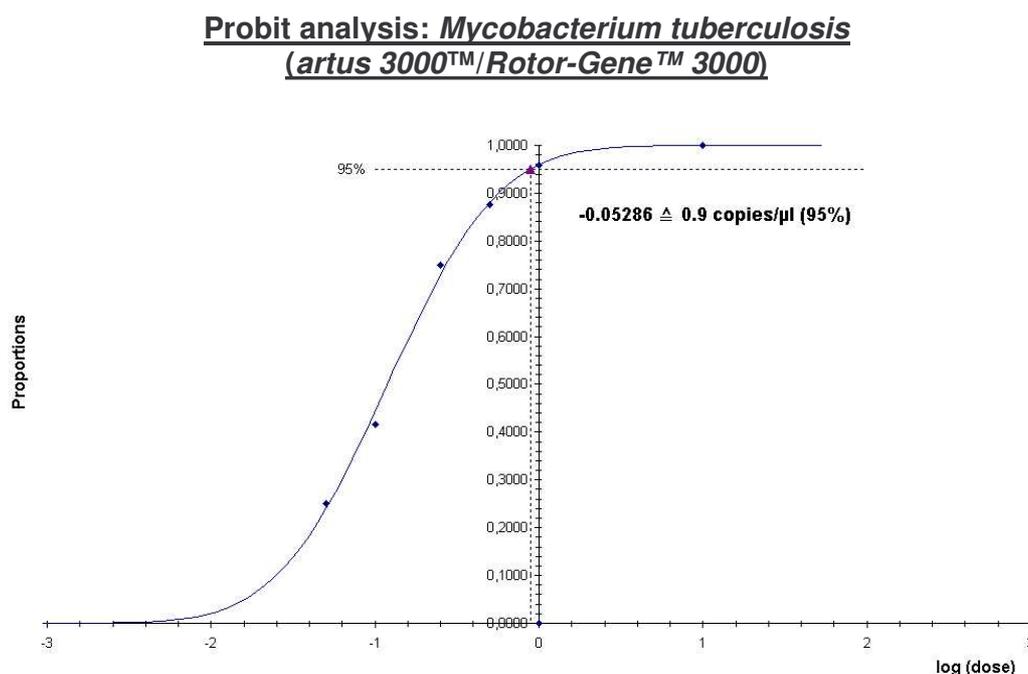


Fig. 10: Analytical sensitivity of the *artus* M. tuberculosis RG PCR Kit.

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<sup>\*</sup> The standard is a cloned PCR product, the concentration of which has been determined by absorption and fluorescence spectroscopy.

## 11.2 Specificity

The specificity of the *artus* M. tuberculosis RG 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 members of the *M. tuberculosis* complex has thus been ensured.

Moreover, the specificity was validated with 90 different *M. tuberculosis* complex negative samples (30 sputum, 30 BAL and 30 bronchial secretion samples). These did not generate any signals with the *M. tuberculosis* complex specific primers and probes, which are included in the *M. tuberculosis* RG Master.

To determine the specificity of the *artus* M. tuberculosis RG 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 (part I): Testing the specificity of the kit with potentially cross-reactive pathogens.

<b>Control Group</b>	<b><i>M. tuberculosis</i> (Cycling A.FAM)</b>	<b><i>Internal Control</i> (Cycling A.JOE)</b>
<i>Actinomyces israelii</i>	-	+
<i>Aeromonas hydrophila</i>	-	+
<i>Bordetella pertussis</i>	-	+
<i>Candida albicans</i>	-	+
<i>Chlamydia trachomatis</i>	-	+
<i>Chlamydia pneumoniae</i>	-	+
<i>Citrobacter freundii</i>	-	+
<i>Corynebacterium diphtheriae</i>	-	+
<i>Corynebacterium jeikeium</i>	-	+
<i>Cryptococcus neoformans</i>	-	+
<i>Eikenella corrodens</i>	-	+
<i>Enterobacter aerogenes</i>	-	+
<i>Enterobacter cloacae</i>	-	+
<i>Enterococcus faecalis</i>	-	+
<i>Enterococcus faecium</i>	-	+
<i>Escherichia coli</i>	-	+
<i>Fusobacterium nucleatum ssp. polymorphum</i>	-	+
<i>Haemophilus influenzae</i>	-	+
<i>Haemophilus parainfluenzae</i>	-	+
<i>Klebsiella pneumoniae</i>	-	+
<i>Lactobacillus acidophilus</i>	-	+
<i>Mycobacterium avium ssp. avium</i>	-	+
<i>Mycobacterium celatum</i>	-	+
<i>Mycobacterium chelonae</i>	-	+
<i>Mycobacterium fortuitum</i>	-	+
<i>Mycobacterium gordonae</i>	-	+
<i>Mycobacterium intracellulare</i>	-	+
<i>Mycobacterium kansasii</i>	-	+
<i>Mycobacterium lentiflavum</i>	-	+
<i>Mycobacterium malmoeense</i>	-	+
<i>Mycobacterium marinum</i>	-	+
<i>Mycobacterium scrofulaceum</i>	-	+
<i>Mycobacterium szulgai</i>	-	+

Table 1 (part II): Testing the specificity of the kit with potentially cross-reactive pathogens.

<b>Control group</b>	<b><i>M. tuberculosis</i> (Cycling A.FAM)</b>	<b><i>Internal Control</i> (Cycling A.JOE)</b>
<i>Mycobacterium ulcerans</i>	-	+
<i>Mycobacterium xenopi</i>	-	+
<i>Neisseria gonorrhoeae</i>	-	+
<i>Neisseria meningitidis</i>	-	+
<i>Nocardia asteroides</i>	-	+
<i>Nocardia brasiliensis</i>	-	+
<i>Nocardia farcinia</i>	-	+
<i>Nocardia otitidiscaviarum</i>	-	+
<i>Peptostreptococcus productus</i>	-	+
<i>Porphyromonas gingivalis</i>	-	+
<i>Prevotella denticola</i>	-	+
<i>Propionibacterium acnes</i>	-	+
<i>Pseudomonas aeruginosa</i>	-	+
<i>Salmonella enteritidis</i>	-	+
<i>Salmonella typhi</i>	-	+
<i>Staphylococcus aureus</i>	-	+
<i>Staphylococcus epidermidis</i>	-	+
<i>Streptococcus agalactiae</i>	-	+
<i>Streptococcus pyogenes</i>	-	+
<i>Streptococcus mutans</i>	-	+
<i>Streptococcus pneumoniae</i>	-	+
<i>Streptomyces venezuelae</i>	-	+
<i>Veillonella parvula</i>	-	+
<i>Xanthomonas maltophilia</i>	-	+

### 11.3 Precision

The precision data of the *artus* M. tuberculosis RG 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* M. tuberculosis RG PCR Kit have been collected using the *Quantitation Standard* of the lowest concentration (QS 4; 30 copies/ $\mu$ l). 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/ $\mu$ l 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 1.26 % (Ct) or 14.64 % (conc.), for the detection of the *Internal Control* 1.57 % (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.

	<b>Standard Deviation</b>	<b>Variance</b>	<b>Coefficient of Variation [%]</b>
Intra-assay variability: <i>M. tuberculosis</i> RG/TM QS 4	0.10	0.01	0.32
Intra-assay variability: <i>Internal Control</i>	0.13	0.02	0.45
Inter-assay variability: <i>M. tuberculosis</i> RG/TM QS 4	0.24	0.06	0.78
Inter-assay variability: <i>Internal Control</i>	0.29	0.08	0.95
Inter-batch variability: <i>M. tuberculosis</i> RG/TM QS 4	0.39	0.15	1.28
Inter-batch variability: <i>Internal Control</i>	0.66	0.43	2.16
Total variance: <i>M. tuberculosis</i> RG/TM QS 4	0.38	0.15	1.26
Total variance: <i>Internal Control</i>	0.48	0.23	1.57

Table 3: Precision data on basis of the quantitative results (in copies/ $\mu$ l).

	<b>Standard deviation</b>	<b>Variance</b>	<b>Coefficient of variation [%]</b>
Intra-assay variability: <i>M. tuberculosis</i> RG/TM QS 4	1.97	3.90	6.56
Inter-assay variability: <i>M. tuberculosis</i> RG/TM QS 4	3.93	15.43	13.00
Inter-batch variability: <i>M. tuberculosis</i> RG/TM QS 4	5.51	30.41	18.09
Total variance: <i>M. tuberculosis</i> RG/TM QS 4	4.44	19.69	14.64

## 11.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus M. tuberculosis* RG PCR Kit. 30 *M. tuberculosis* complex negative samples of each sputum, BAL and bronchial secretion were spiked with 3 copies/ $\mu$ l elution volume of *M. tuberculosis* control DNA (approx. threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DNA Mini Kit (see **8.1 DNA Isolation**) these samples were analyzed with the *artus M. tuberculosis* RG PCR Kit. For all *M. tuberculosis* samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of *M. tuberculosis* complex negative sputum, BAL and bronchial secretion samples (30 each). The total failure rate was 0 %. Inhibitions were not observed. Thus, the robustness of the *artus M. tuberculosis* RG PCR Kit is  $\geq 99$  %.

## 11.5 Reproducibility

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

## 12. Product Use Limitations

- The *artus M. tuberculosis* RG 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.

## **13. Safety information**

For safety information of the *artus* M.tuberculosis RG PCR Kit, please consult the appropriate material safety data sheet (MSDS). The MSDS are available online in convenient and compact PDF format at [www.qiagen.com/support/msds.aspx](http://www.qiagen.com/support/msds.aspx).

## **14. Quality control**

In accordance with QIAGEN's ISO 9001 and ISO 13485-certified Quality Management System, each lot of *artus* M.tuberculosis RG 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



Contains sufficient for <N> tests



Temperature limitation

**QS**

*Quantitation Standard*

**IC**

*Internal Control*

**Mg-Sol**

*Magnesium Solution*



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