artus® Parvo B19 RG PCR Kit

Handbook

Σ 24 (catalog no. 4504263)

Quantitative in vitro Diagnostics

For use with the *Rotor-Gene*® *Q instrument*

July 2018 – Version 1

CE

IVD

REF 4504263, 4504265

HB 1112933 EN

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

R4 MAT 1112933 EN

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 Parvo B19 RG PCR Kit

For use with the *Rotor-Gene Q instrument*.

1. Contents

	Labelling and contents	Cat. no. 4504263 24 reactions
Blue	*Parvo B19 RG/TM Master*	2 x 12 rxns
Red	*Parvo B19 RG/TM QS 1*[a] *1 x 10*^{5} *IU/µl*	1 x 200 µl
Red	*Parvo B19 RG/TM QS 2*[a] *1 x 10*^{4} *IU/µl*	1 x 200 µl
Red	*Parvo B19 RG/TM QS 3*[a] *1 x 10*^{3} *IU/µl*	1 x 200 µl
Red	*Parvo B19 RG/TM QS 4*[a] *1 x 10*^{2} *IU/µl*	1 x 200 µl
Red	*Parvo B19 RG/TM QS 5*[a] *1 x 10*^{1} *IU/µl*	1 x 200 µl
Green	*Parvo B19 RG/TM IC*[a]	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* Parvo B19 RG 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
- DNA isolation kit (see **Section 9.1 DNA Isolation**)
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- *Rotor-Gene Q instrument* with software version 2.3 or later
- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106)
- Cooling block (Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901General 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 (72well loading block).

4. Intended Use

The artus Parvo B19 RG PCR Kit is an in vitro nucleic acid amplification test for the detection and quantification of parvovirus B19 DNA in human serum or EDTA plasma.The kit utilizes real time polymerase chain reaction (PCR) and is configured for use with the QIAamp UltraSens Virus Kit, QIAamp DNA Mini Kit and the Rotor-Gene Q instrument.

The kit is not intended to be used as a blood/blood product screening test for parvovirus B19 infection The artus Parvo B19 RG PCR Kit is intended for in vitro diagnostic use by healthcare professionals.

5. Pathogen Information

The majority of parvovirus B19 infections are clinically asymptomatic. The symptoms of an acute infection with parvovirus B19 are flu-like, but may also resemble those of rubella (German measles) and, especially in adults, those of rheumatism. Parvovirus B19 is a major cause of a plastic crisis in patients with hemolytic anemia. Severe fetal complications are sometimes observed, especially following maternal infections during the second and third trimesters.

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* Parvo B19 RG PCR Kit constitutes a ready-to-use system for the detection of parvovirus B19 DNA using polymerase chain reaction (PCR) in the *Rotor-Gene Q instrument*. The *Parvo B19 RG/TM Master* contains reagents and enzymes for the specific amplification of a 76 bp region of the parvovirus B19 genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling A.Green of the *Rotor-Gene Q instrument*. In addition, the *artus* Parvo B19 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.Yellow. The detection limit of the analytical parvovirus B19 PCR (see **section 12.1 Analytical Sensitivity**) is not reduced. External positive controls (*Parvo B19 RG/TM QS 1 – 5*) are supplied which allow the determination of the pathogen load. For further information, please refer to **section 9.3 Quantitation**.

8. Protocol

8.1 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 kits are recommended:

Sample Material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer	Carrier RNA
Serum, plasma	QIAamp® UltraSens® Virus Kit (50)	53 704	QIAGEN	included
	QIAamp DNA Mini Kit (50)	51 304	QIAGEN	not included

- 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 lyophilized carrier RNA using the elution buffer (do not use lysis buffer) of the extraction kit (e.g., Buffer AE 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 –15°C to –30°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 **section 9.2 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., 2400 µl
Carrier RNA (1 µg/µl)	2 µl	24 µl
Total Volume	**202 µl**	**2424 µl**
Volume per extraction	**200 µl**	**each 200 µl**

c) Please use the freshly prepared mixture of lysis buffer and carrier RNA instantly for extraction. Storage of the mixture is not possible.

- 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 UltraSens Virus 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 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 –15°C to –30°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 **section 9.2 Internal Control**) should be prepared freshly according to the following pipetting scheme:

Number of samples	1	12
Lysis buffer AC	800 µl	9600 µl
Carrier RNA (1 µg/µl)	5.6 µl	67.2 µl
Total Volume	**805.6 µl**	**9667.2 µl**
Volume per extraction	**800 µl**	**each 800 µl**

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

- It is recommended to elute the DNA in 50 µl elution buffer to get the highest sensitivity of the *artus* Parvo B19 RG PCR Kit.
- The **QIAamp UltraSens Virus Kit** allows a sample concentration. If you use sample material other than serum or plasma, please add at least 50 % (v/v) of negative human plasma to the sample.
- 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* Parvo B19 RG PCR Kit should not be used with **phenol**-based isolation methods.

Important: The *Internal Control* of the *artus* Parvo B19 RG PCR Kit can be used directly in the isolation procedure (see **section 9.2 Internal Control**).

8.2 Internal Control

An *Internal Control* (*Parvo B19 RG/TM 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 UltraSens Virus Kit the DNA is eluted in 50 µl Buffer AVE. Hence, 5 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 **section 9.1 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 2 µl of the *Internal Control* per reaction directly to 30 µl *Parvo B19 RG/TM Master*. For each PCR reaction use 30 µl of the Master Mix produced as described above* and add 20 µl of the purified sample. If you are preparing a PCR run for several samples please increase the volume of the *Parvo B19 RG/TM Master* and the *Internal Control* according to the number of samples (see **section 9.4 Preparing the PCR**).

8.3 Quantitation

The enclosed *Quantitation Standards* (*Parvo B19 RG/TM QS 1 – 5*) are treated as previously purified samples and the same volume is used (20 µl). To generate a standard curve on the *Rotor-Gene Q instrument*, all five *Quantitation Standards* should be used and defined in the menu window *Edit Samples* as standards with the specified concentrations (see *Rotor-Gene Q User Manual*. 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 *Rotor-Gene* Q *User Manual*). 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 IU/µl. The following equation has to be applied to convert the values determined using the standard curve into IU/ml of sample material:

* 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.

$$\text{Result in sample material (IU/ml)} = \frac{\text{Result in eluate (IU/µl) x Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

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).

8.4 Preparing the PCR

Make sure that the Cooling Block (accessory of the *Rotor-Gene Q instrument*) 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* (*Parvo B19 RG/TM QS 1 – 5*) 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 monitor the DNA isolation procedure and to check for possible PCR inhibition,** it has already been added to the isolation (see **section 9.2 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	*Parvo B19 RG/TM Master*	30 µl	360 µl
	Parvo B19 RG/TM IC	0 µl	0 µl
	Total Volume	**30 µl**	**360 µl**
2. Preparation of PCR assay	Master Mix	30 µl	30 µl each
	Sample	20 µl	20 µl each
	Total Volume	**50 µl**	**50 µl each**

If you want to use the *Internal Control* **exclusively to check for PCR inhibition**, it must be added directly to the *Parvo B19 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	*Parvo B19 RG/TM Master*	30 µl	360 µl
	Parvo B19 RG/TM IC	2 µl	24 µl
	Total Volume	**32 µl***	**384 µl**
2. Preparation of PCR assay	Master Mix	30 µl	30 µl each
	Sample	20 µl	20 µl each
	Total Volume	**50 µl**	**50 µl each**

Pipette 30 µl of the Master Mix into each PCR tube. Then add 20 µl of the eluted sample DNA to each tube and mix well by pipetting up and down several times. Correspondingly, 20 µl of at least one of the *Quantitation Standards* (*Parvo B19 RG/TM QS 1 – 5*) must be used as a positive control and 20 µl of water (*Water*, *PCR grade*) as a negative control. Close the PCR tubes. Please take care that the *Locking Ring* (accessory of the *Rotor-Gene Q instrument*) is placed on top of the rotor to prevent accidental opening of the tubes during the run.

* 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

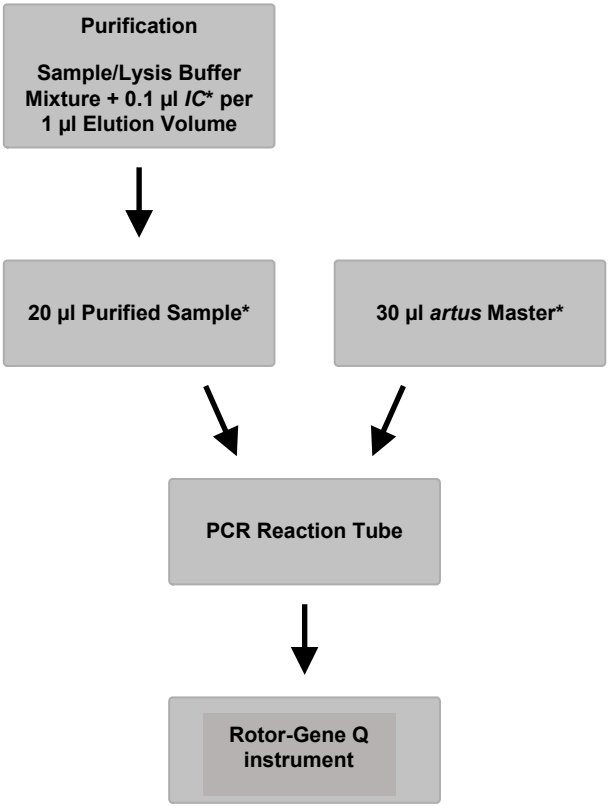

Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

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

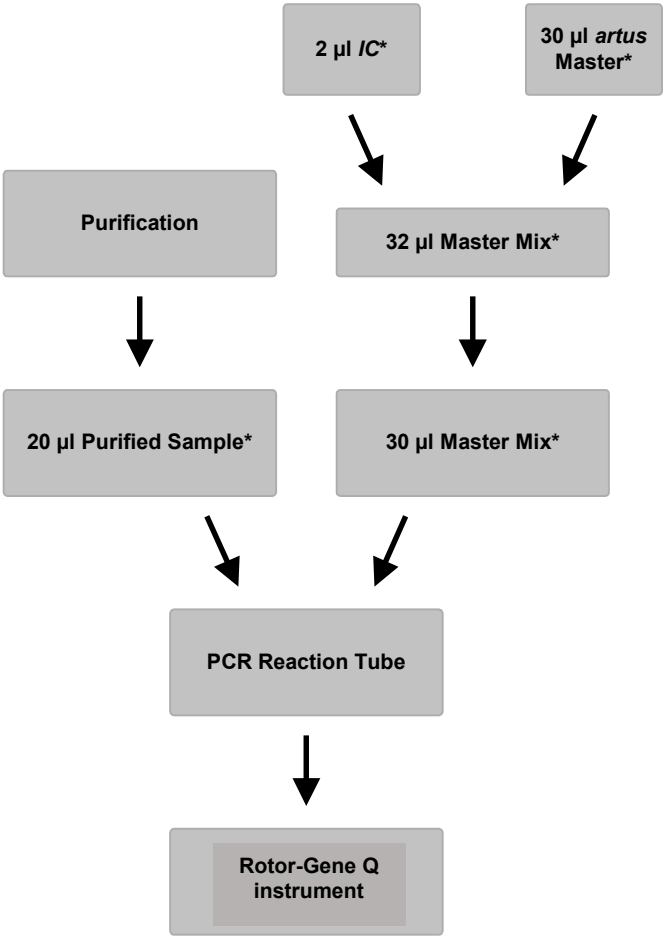

Fig. 2: Schematic workflow for the control of PCR inhibition.

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

8.5 Programming of the *Rotor-Gene Q instrument*

For the detection of Parvo B19 DNA, create a temperature profile on your *Rotor-Gene Q instrument* 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 *Rotor-Gene Q instrument* Run	Fig. 7

All specifications refer to the *Rotor-Gene* software version 2.3. Please find further information on programming the *Rotor-Gene Q instrument* in the *Rotor-Gene Q User Manual.*

First, elect “Empty Run” in the Advanced tab of the “New Run” dialog box. In the “Rotor Type” Panel, select “72-Well Rotor”, check the “Locking Ring Attached” box and click “Next”.

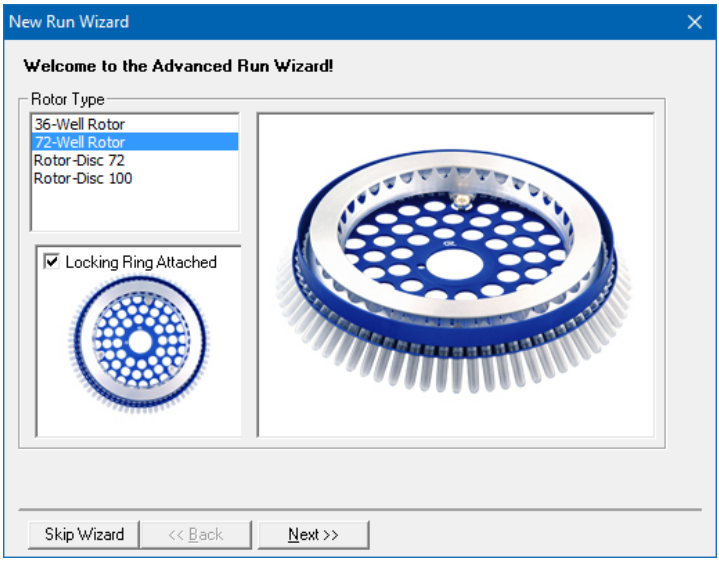

Fig. 3: New run wizard welcome screen.

Then, enter PCR reaction volume in the next menu window *New Run Wizard* (see Fig. 4).

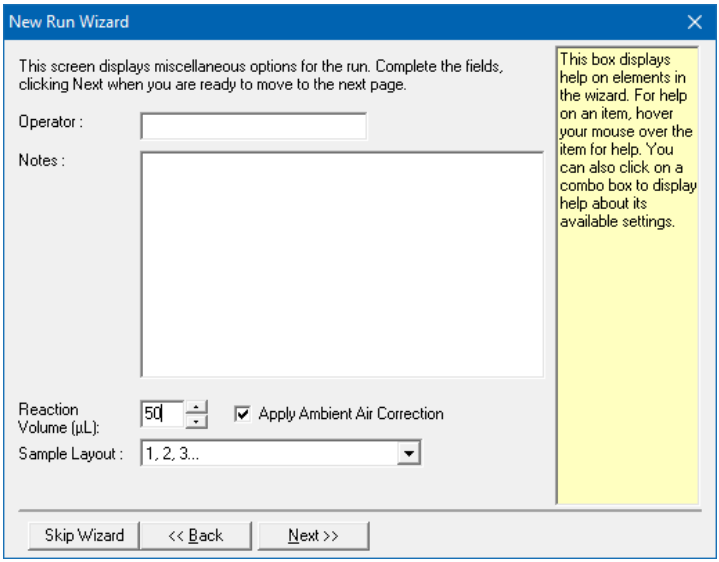

Fig. 4: Setting of General Assay Parameters.

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

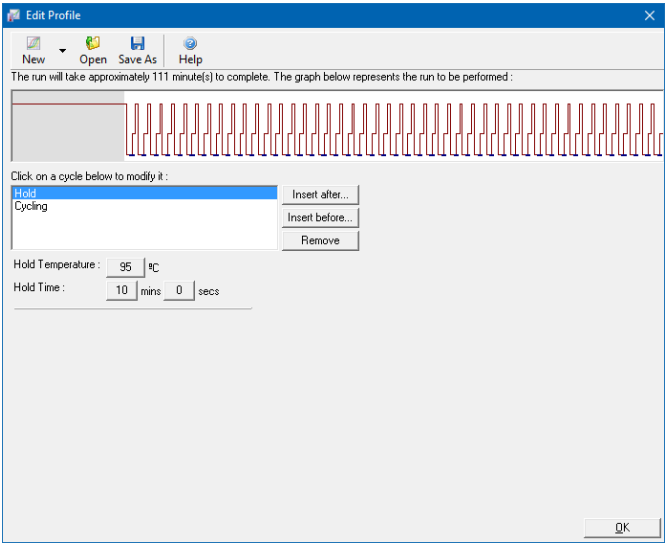

Fig. 5: Initial Activation of the Hot Start Enzyme.

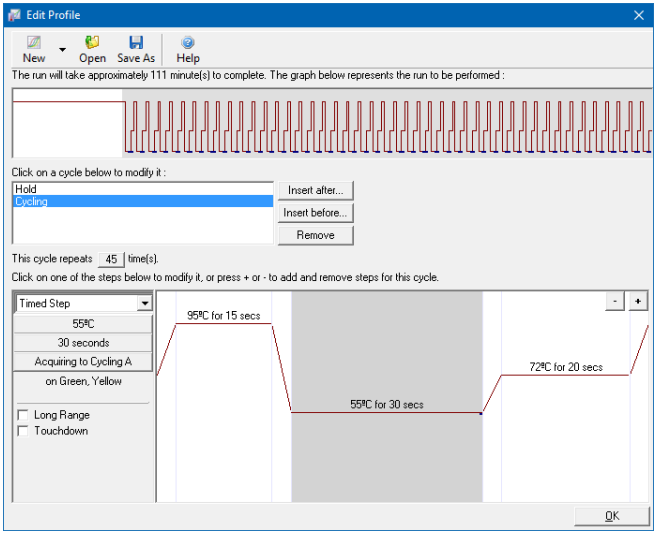

Fig. 6: Amplification of the DNA.

The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. This adjustment is performed in the menu window *Auto Gain Optimisation Setup* (activation in menu window *New Run Wizard* under *Gain Optimisation*). Please set the calibration temperature to the annealing temperature of the amplification program (see Fig. 7), select “Optimise Acquiring” and start the procedure.

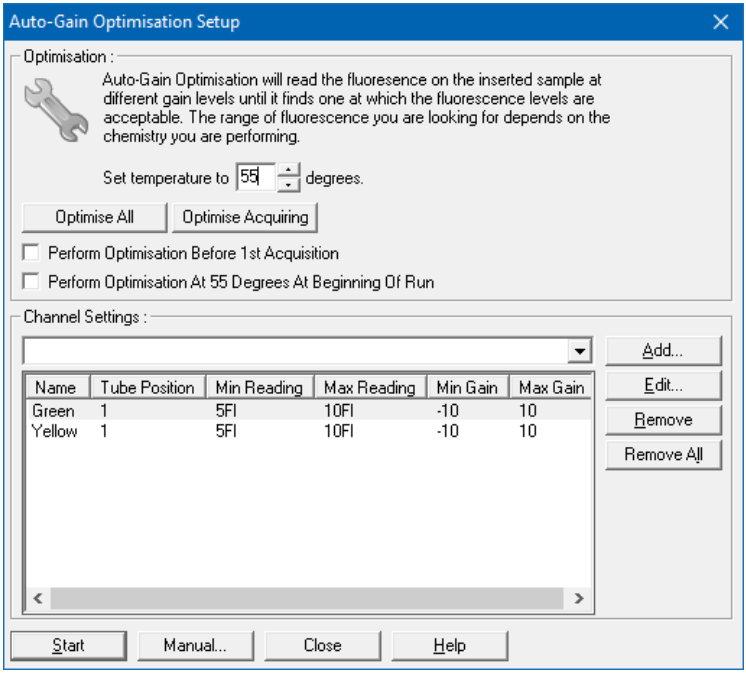

Fig. 7: Adjustment of the Fluorescence Channel Sensitivity.

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

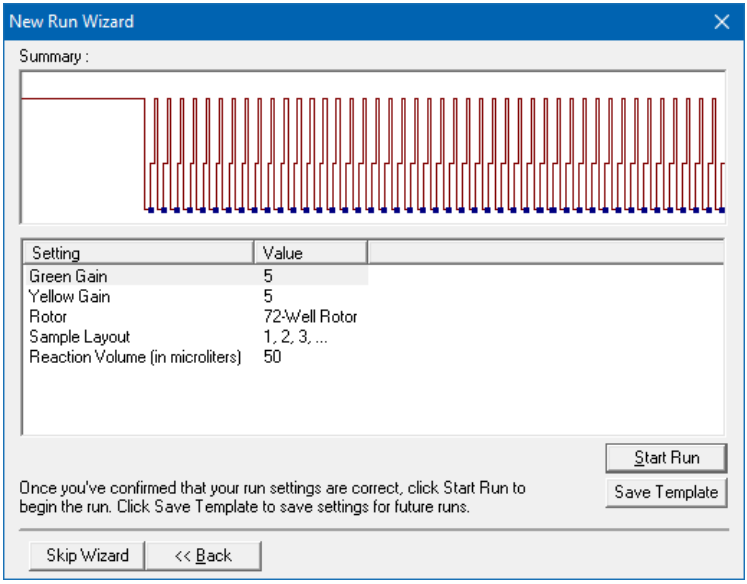

Fig. 8: Starting of the *Rotor-Gene Q instrument* Run.

9. Data Analysis

Data analysis is performed with the *Rotor-Gene* software according to the manufacturer's instructions (*Rotor-Gene Q User Manual*).

The following results are possible:

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

   The result of the analysis is positive: The sample contains parvovirus B19 DNA.

   In this case, the detection of a signal in the Cycling A.Yellow channel is dispensable, since high initial concentrations of parvovirus B19 DNA (positive signal in the Cycling A.Green channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the Cycling A.Yellow channel (competition).

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

   In the sample no parvovirus B19 DNA is detectable. It can be considered negative.

   In the case of a negative parvovirus B19 PCR the detected signal of the *Internal Control* rules out the possibility of PCR inhibition.

3. No signal is detected in the Cycling A.Green or in the Cycling A.Yellow channel.

   No result can be concluded.

   Information regarding error sources and their solution can be found in **section 11. Troubleshooting**.

Examples of positive and negative PCR reactions are given in Fig. 9 and Fig. 10.

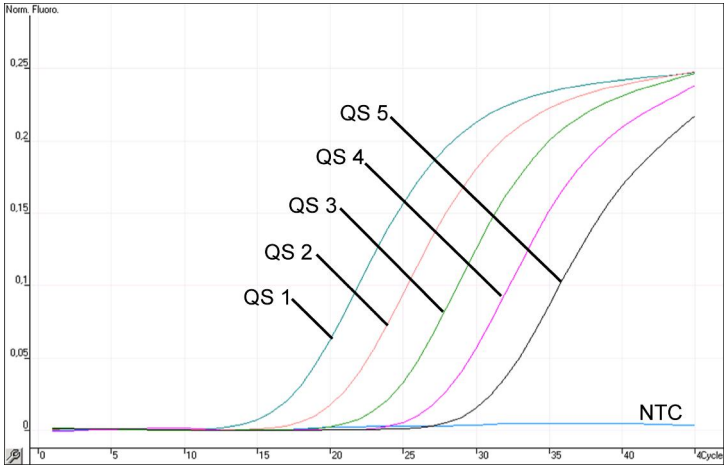

Fig. 9: Detection of the *Quantitation Standards* (*Parvo B19 RG/TM QS 1 – 5*) in fluorescence channel Cycling A.Green. NTC: non-template control (negative control).

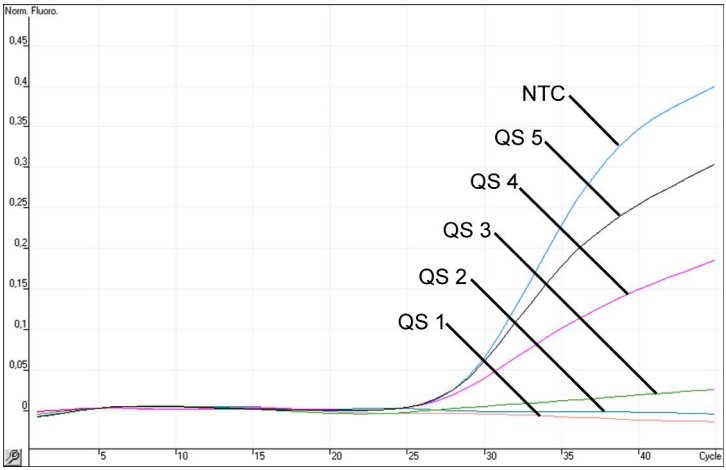

Fig. 10: Detection of the *Internal Control* (*IC*) in fluorescence channel Cycling A.Yellow with simultaneous amplification of the *Quantitation Standards* (*Parvo B19 RG/TM QS 1 – 5*). NTC: non-template control (negative control).

10. Troubleshooting

No signal with positive controls (*Parvo B19 RG/TM QS 1 – 5*) in fluorescence channel Cycling A.Green:

- The selected fluorescence channel for PCR data analysis does not comply with the protocol.
  - → For data analysis select the fluorescence channel A.Green for the analytical parvovirus B19 PCR and the fluorescence channel A.Yellow for the *Internal Control* PCR
- Incorrect programming of the temperature profile of the *Rotor-Gene Q instrument*.
  - → Compare the temperature profile with the protocol (see **section 9.5 Programming of the *Rotor-Gene Q instrument***).

- Incorrect configuration of the PCR reaction.
  - → Check your work steps by means of the pipetting scheme (see **section 9.4 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 **section 2. Storage** or the *artus* Parvo B19 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.Yellow and simultaneous absence of a signal in channel Cycling A.Green:

- 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 **section 9.1 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 **section 9.1 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 **section 9.1 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 **section 2. Storage** or the *artus* Parvo B19 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.Green 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 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* Parvo B19 RG PCR Kit, a standard dilution series has been set up from 100 to nominal 0.03 parvovirus B19 IU*/µl and analysed with the *artus* Parvo B19 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. 11. The analytical detection limit of the *artus* Parvo B19 RG PCR Kit is 0.2 IU/µl (p = 0.05). This means that there is a 95 % probability that 0.2 IU/µl will be detected.

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

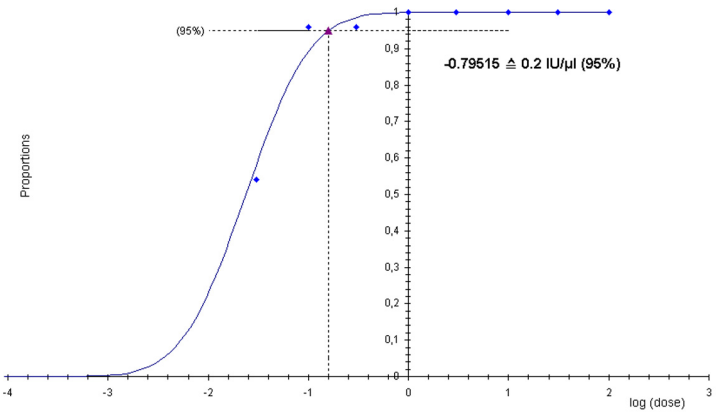

Fig. 11: Analytical sensitivity of the *artus* Parvo B19 RG PCR Kit.

11.2 Specificity

The specificity of the *artus* Parvo B19 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 relevant genotypes has thus been ensured.

Moreover, the specificity was validated with six different parvovirus B19 negative serum samples. These did not generate any signals with the parvovirus B19 specific primers and probes, which are included in the *Parvo B19 RG/TM Master*.

To determine the specificity of the *artus* Parvo B19 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: Testing the specificity of the kit with potentially cross-reactive pathogens.

Control group	Parvovirus B19 (Cycling A.Green)	*Internal Control* (Cycling A.Yellow)
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 T cell leukaemia virus 1	–	+
Human T cell leukaemia virus 2	–	+

11.3 Precision

The precision data of the *artus* Parvo B19 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* Parvo B19 RG PCR Kit have been collected using the *Quantitation Standard* of the lowest concentration (*QS 5*; 10 IU/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 IU/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 1.66 % (Ct) or 17.65 % (conc.), for the detection of the

Internal Control 0.90 % (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: *Parvo B19 RG/TM QS 5*	0.22	0.05	0.75
Intra-assay variability: *Internal Control*	0.18	0.03	0.80
Inter-assay variability: *Parvo B19 RG/TM QS 5*	0.32	0.10	1.11
Inter-assay variability: *Internal Control*	0.19	0.03	0.84
Inter-batch variability: *Parvo B19 RG/TM QS 5*	0.38	0.14	1.47
Inter-batch variability: *Internal Control*	0.21	0.04	0.92
Total variance: *Parvo B19 RG/TM QS 5*	0.48	0.23	1.66
Total variance: *Internal Control*	0.20	0.04	0.90

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

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: *Parvo B19 RG/TM QS 5*	0.96	0.93	9.58
Inter-assay variability: *Parvo B19 RG/TM QS 5*	1.33	1.78	13.22
Inter-batch variability: *Parvo B19 RG/TM QS 5*	2.27	5.17	22.20
Total variance: *Parvo B19 RG/TM QS 5*	1.79	3.21	17.65

11.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* Parvo B19 RG PCR Kit. 30 parvovirus B19 negative samples of serum were spiked with 1 IU/µl elution volume of parvovirus B19 control DNA (fivefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DNA Mini Kit (see **section 9.1 DNA Isolation**) these samples were analysed with the *artus* Parvo B19 RG PCR Kit. For all parvovirus B19 samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 30 parvovirus B19 negative serum samples. The total failure rate was 0 %. Inhibitions were not observed. Thus, the robustness of the *artus* Parvo B19 RG PCR Kit is ≥ 99 %.

11.5 Reproducibility

Reproducibility data permit a regular performance assessment of the artus Parvo B19 RG PCR Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programs.

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.
- For some genotype 3–related sequences the claimed performance cannot be guaranteed. Due to mutations in the primer/probe binding region a significant decrease of sensitivity could occur (Baylis and Buchheit, 2009).

- 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. Warnings and Precautions

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 ISO-certified Quality Management System, each lot of *artus* Parvo B19 RG PCR Kit has been tested against predetermined specifications to ensure consistent product quality.

15. References

Baylis SA, Buchheit KH. A proficiency testing study to evaluate laboratory performance for the detection of different genotypes of parvovirus B19. Vox Sang. 2009; 97 (1): 13 – 20.

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

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*

artus Parvo B19 RG PCR Kit

Trademarks and Disclaimers
QIAGEN®, QIAamp®, *artus*®, Rotor-Gene®, UltraSens® (QIAGEN Group).

Document Revision History	
R4 07/2018	This is revision 4 of the handbook for the *artus* Parvo RG PCR Kit. Changes from the previous version include clarification on the intended use and updating the description of the Rotor-Gene Q instrument and software to the versions that are current available.

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

The *artus* Parvo B19 RG PCR Kit is a CE-marked diagnostic kit according to the European In Vitro Diagnostic Directive 98/79/EC. Not available in all countries.

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.

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.

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the *artus* Parvo B19 RG PCR Kit to the following terms:

1. The *artus* Parvo B19 RG PCR Kit may be used solely in accordance with the artus *Parvo B19 RG 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 *Parvo B19 RG 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** .

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)

1112933

QIAGEN

Sample & Assay Technologies