

artus[®] Parvo B19 LC PCR Kit

Handbook



24 (catalog no. 4504063)



96 (catalog no. 4504065)

Quantitative in vitro Diagnostics

For use with the *LightCycler*[®] Instrument

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artus Parvo B19 LC PCR Kit

For use with the *LightCycler* Instrument.

1. Contents

	Labelling and contents	Art. No. 4504063 24 reactions	Art. No. 4504065 96 reactions
Blue	<i>Parvo B19 LC Master</i>	2 x 12 rxns	8 x 12 rxns
Yellow	<i>Parvo B19 LC Mg-Sol[¶]</i>	1 x 400 µl	1 x 400 µl
Red	<i>Parvo B19 LC QS 1[¶]</i> <i>1 x 10⁵ IU/µl</i>	1 x 200 µl	1 x 200 µl
Red	<i>Parvo B19 LC QS 2[¶]</i> <i>1 x 10⁴ IU/µl</i>	1 x 200 µl	1 x 200 µl
Red	<i>Parvo B19 LC QS 3[¶]</i> <i>1 x 10³ IU/µl</i>	1 x 200 µl	1 x 200 µl
Red	<i>Parvo B19 LC QS 4[¶]</i> <i>1 x 10² IU/µl</i>	1 x 200 µl	1 x 200 µl
Red	<i>Parvo B19 LC QS 5[¶]</i> <i>1 x 10¹ IU/µl</i>	1 x 200 µl	1 x 200 µl
Green	<i>Parvo B19 LC IC[¶]</i>	1 x 1000 µl	2 x 1000 µl
White	<i>Water (PCR grade)</i>	1 x 1000 µl	1 x 1000 µl

- [¶] QS = Quantitation Standard
IC = Internal Control
Mg-Sol = Magnesium Solution

2. Storage

The components of the *artus Parvo B19 LC 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 $+4^{\circ}\text{C}$ 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
- *Color Compensation Set* (Roche Diagnostics, Cat. No. 2 158 850) for the installation of a *Crosstalk Color Compensation* file
- *LightCycler* Capillaries (20 µl)
- *LightCycler* Cooling Block
- *LightCycler* Instrument
- *LightCycler* Capping Tool

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 *LightCycler* Cooling Block.

5. Intended Use

The *artus* Parvo B19 LC 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 Roche LightCycler 1.1/1.2/1.5/2.0 instruments.

The kit is not intended to be used as a blood/blood product screening test for parvovirus B19 infection.

The *artus* Parvo B19 LC PCR Kit is intended for in vitro diagnostic use by healthcare professionals.

6. 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 aplastic crisis in patients with hemolytic anemia. Severe fetal complications are sometimes observed, especially following maternal infections during the second and third trimesters.

7. Principle of Real-Time PCR

Pathogen diagnosis using 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).

8. Product Description

The *artus* Parvo B19 LC PCR Kit constitutes a ready-to-use system for the detection of parvovirus B19 DNA using PCR in the *LightCycler* Instrument. The *Parvo B19 LC Master* contains reagents and enzymes for the specific amplification of a 259 bp region of the parvovirus B19 genome, and for the direct detection of the specific amplicon in fluorimeter channel F2 of the

LightCycler Instrument. In addition, the *artus* Parvo B19 LC PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an *Internal Control (IC)* in fluorimeter channel F3. The detection limit of the analytical parvovirus B19 PCR (see **section 12.1 Analytical Sensitivity**) is not reduced. External positive controls (*Parvo B19 LC QS 1 – 5*) are supplied which allow the determination of the pathogen load. For further information, please refer to **section 9.3 Quantitation**.

9. Protocol

9.1 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 -20°C . Avoid repeated thawing ($> 2 \times$) of a carrier RNA aliquot.

- b) Use $1 \mu\text{g}$ carrier RNA per $100 \mu\text{l}$ lysis buffer. For instance, if the extraction protocol suggests $200 \mu\text{l}$ lysis buffer, please add $2 \mu\text{l}$ carrier RNA ($1 \mu\text{g}/\mu\text{l}$) 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., $200 \mu\text{l}$	e.g., $2400 \mu\text{l}$
Carrier RNA ($1 \mu\text{g}/\mu\text{l}$)	$2 \mu\text{l}$	$24 \mu\text{l}$
Total Volume	$202 \mu\text{l}$	$2424 \mu\text{l}$
Volume per extraction	$200 \mu\text{l}$	each $200 \mu\text{l}$

- 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 $310 \mu\text{l}$ of the elution buffer provided with the kit (final concentration $1 \mu\text{g}/\mu\text{l}$, 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 ($> 2 \times$) 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	9,600 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 LC 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 LC PCR Kit should not be used with **phenol**-based isolation methods.

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

9.2 Internal Control

An *Internal Control* (*Parvo B19 LC 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 AVE buffer. 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 0.5 µl of the *Internal Control* and 2 µl *Parvo B19 LC Mg-Sol* per reaction directly to 13 µl *Parvo B19 LC Master*. For each PCR reaction use 15 µl of the Master Mix produced as described above* and add 5 µl of the purified sample. If you are preparing a PCR run for several samples please increase the volume of the *Parvo B19 LC Master*, the *Parvo B19 LC Mg-Sol* and the *Internal Control* according to the number of samples (see **section 9.4 Preparing the PCR**).

9.3 Quantitation

The enclosed *Quantitation Standards (Parvo B19 LC QS 1 – 5)* are treated as previously purified samples and the same volume is used (5 µl). To generate a standard curve on the *LightCycler* Instrument, all five *Quantitation Standards* should be used and defined in the *Sample Loading Screen* as standards with the specified concentrations (see *LightCycler Operator's Manual*, Version 3.5, Chapter B, 2.4. Sample Data Entry). The standard curve generated as above can also be used for subsequent runs, provided that at least one standard of **one** given concentration is used in the current run. For this purpose, the previously generated standard curve needs to be imported (see *LightCycler Operator's Manual*, Version 3.5, Chapter B, 4.2.5. Quantitation with an External Standard Curve). However, this quantitation method may lead to deviations in the results due to variability between different PCR runs.

* 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.

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:

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

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).

Important: A guideline for the quantitative analysis of *artus* systems on the *LightCycler* Instruments are provided at <https://www.qiagen.com/TechnicalNoteLightCycler1> for the LightCycler 1.1/1.2/1.5 and at <https://www.qiagen.com/TechnicalNoteLightCycler2> for the LightCycler 2.0.

9.4 Preparing the PCR

Make sure that the Cooling Block as well as the capillary adapters (accessories of the *LightCycler* Instrument) are pre-cooled to +4°C. Place the desired number of *LightCycler* capillaries into the adapters of the Cooling Block. Please make sure that at least one *Quantitation Standard* as well as one negative control (*Water, PCR grade*) are included per PCR run. To generate a standard curve, use all supplied *Quantitation Standards* (*Parvo B19 LC 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	<i>Parvo B19 LC Master</i>		13 µl	156 µl
	<i>Parvo B19 LC Mg-Sol</i>		2 µl	24 µl
	<i>Parvo B19 LC IC</i>		0 µl	0 µl
	Total Volume		15 µl	180 µl
2. Preparation of PCR assay	Master Mix		15 µl	15 µl each
	Sample		5 µl	5 µl each
	Total Volume		20 µl	20 µl each

If you want to use the *Internal Control* **exclusively to check for PCR inhibition**, it must be added directly to the *Parvo B19 LC Master*. In this case, please use the following pipetting scheme (for a schematic overview see Fig. 2):

	Number of samples		1	12
1. Preparation of Master Mix	<i>Parvo B19 LC Master</i>		13 µl	156 µl
	<i>Parvo B19 LC Mg-Sol</i>		2 µl	24 µl
	<i>Parvo B19 LC IC</i>		0.5 µl	6 µl
	Total Volume		15 µl*	186 µl
2. Preparation of PCR assay	Master Mix		15 µl	15 µl each
	Sample		5 µl	5 µl each
	Total Volume		20 µl	20 µl each

Pipette 15 µl of the Master Mix into the plastic reservoir of each capillary. Then add 5 µl of the eluted sample DNA. Correspondingly, 5 µl of at least one of the *Quantitation Standards (Parvo B19 LC QS 1 – 5)* must be used as a positive control and 5 µl of water (*Water, PCR grade*) as a negative control. Close the capillaries. To transfer the mixture from the plastic reservoir into the capillary, centrifuge the adapters containing the capillaries in a desktop centrifuge for ten seconds at a maximum of 400 x g (2000 rpm).

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

Addition of the *Internal Control* to the Purification Procedure

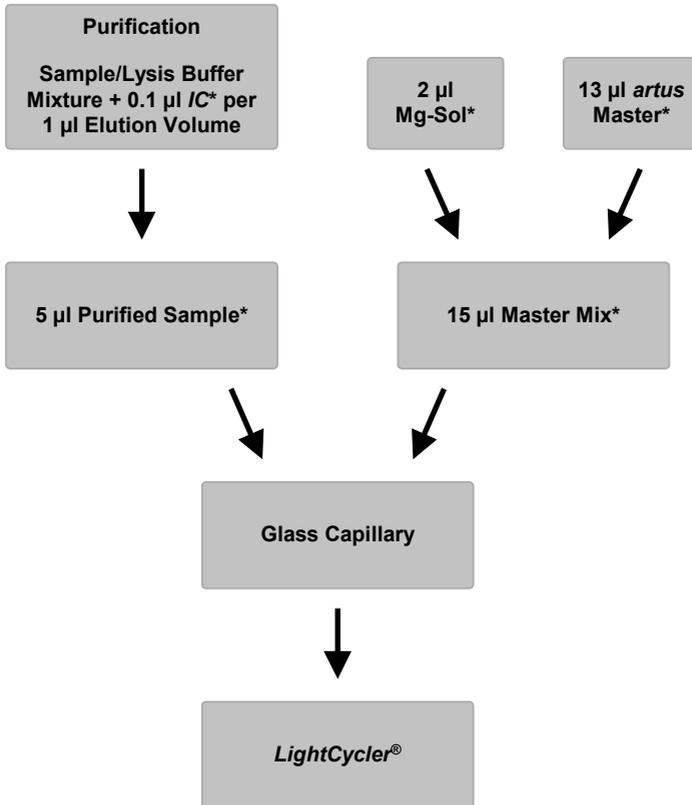


Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

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

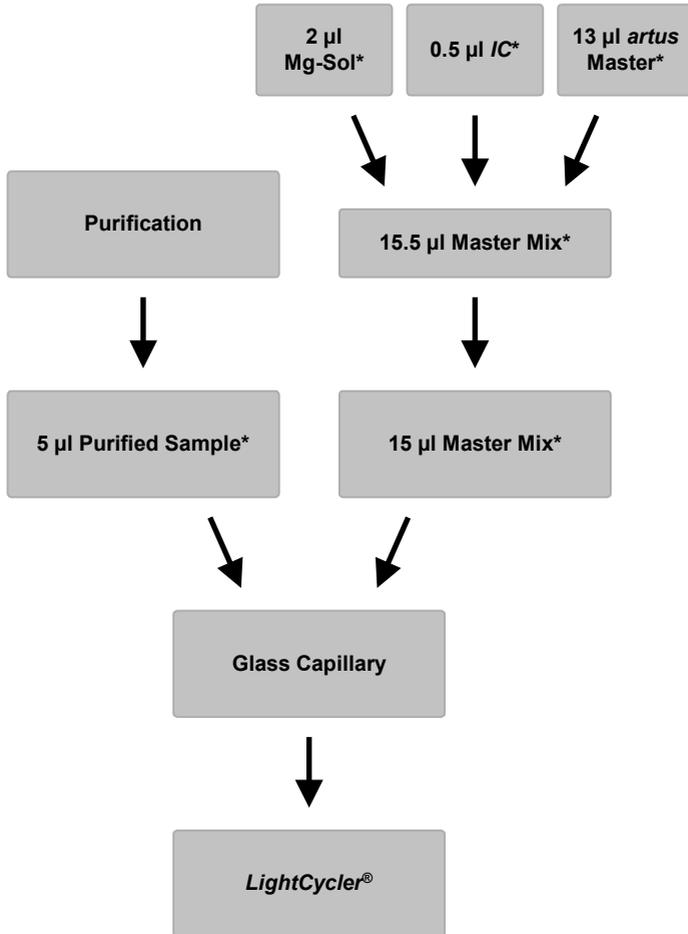


Fig. 2: Schematic workflow for the control of PCR inhibition.

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

9.5 Programming of the *LightCycler* Instrument

For the detection of parvovirus B19 DNA, create a temperature profile on your *LightCycler* Instrument according to the following three steps (see Fig. 3 - 5).

- A. Initial Activation of the Hot Start Enzyme Fig. 3
- B. Amplification of the DNA Fig. 4
- C. Cooling Fig. 5

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

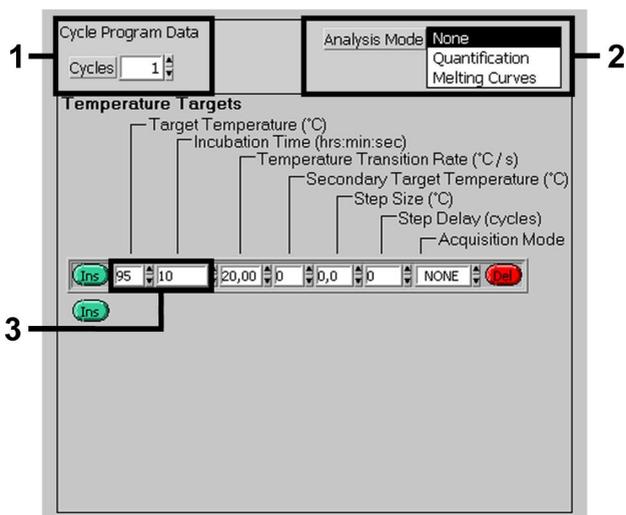


Fig. 3: Initial Activation of the Hot Start Enzyme.

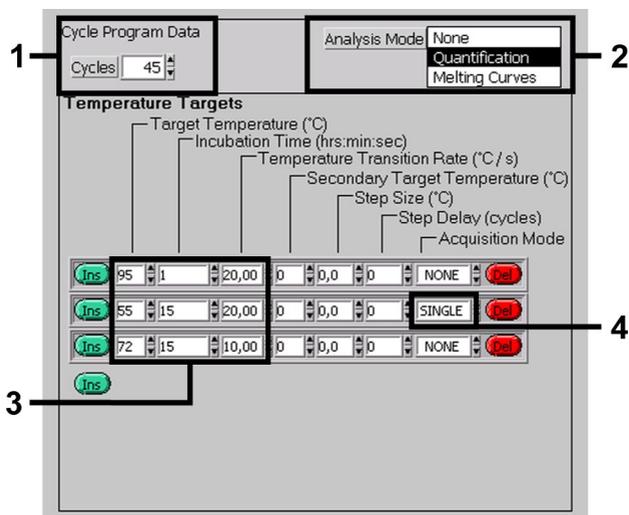


Fig. 4: Amplification of the DNA.

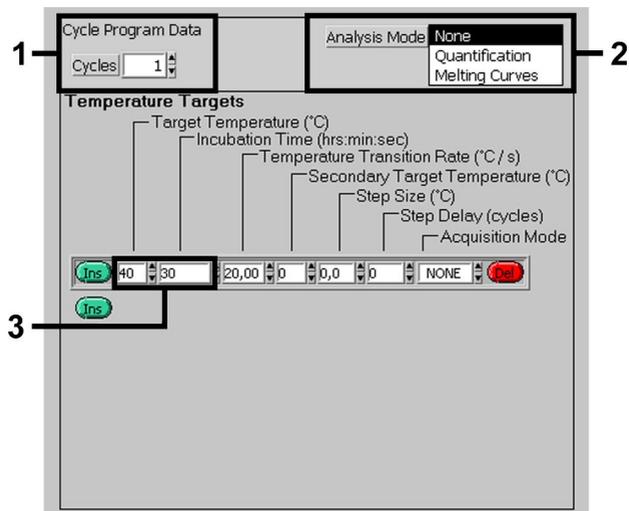


Fig. 5: Cooling.

10. Data Analysis

In multicolor analyses interferences occur between fluorimeter channels. The *LightCycler* Instrument's software contains a file termed *Color Compensation File*, which compensates for these interferences. Open this file before, during or after the PCR run by activating the *Choose CCC File* or the *Select CC Data* button. If no *Color Compensation File* is installed, generate the file according to the instructions in the *LightCycler Operator's Manual*. After the *Color Compensation File* has been activated, separate signals appear in fluorimeter channels F1, F2 and F3. For analysis of the PCR results gained with the *artus Parvo B19 LC PCR Kit* please select fluorescence display options F2/Back-F1 for the analytical parvovirus B19 PCR and F3/Back-F1 for the *Internal Control* PCR, respectively. For the analysis of quantitative runs, please follow the instructions given in **section 9.3 Quantitation** and in the Technical Notes, which are provided at the following web addresses: <https://www.qiagen.com/TechnicalNoteLightCycler1> for the LightCycler 1.1/1.2/1.5 and <https://www.qiagen.com/TechnicalNoteLightCycler2> for the LightCycler 2.0.

The following results are possible:

1. A signal is detected in fluorimeter channel F2/Back-F1.

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

In this case, the detection of a signal in the F3/Back-F1 channel is dispensable, since high initial concentrations of parvovirus B19 DNA (positive signal in the F2/Back-F1 channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the F3/Back-F1 channel (competition).

2. In fluorimeter channel F2/Back-F1 no signal is detected. At the same time, a signal from the *Internal Control* appears in the F3/Back-F1 channel.

In the sample no 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 F2/Back-F1 or in the F3/Back-F1 channel.

No diagnosis can be concluded.

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

Examples of positive and negative PCR reactions are given in Fig. 6 and Fig. 7.

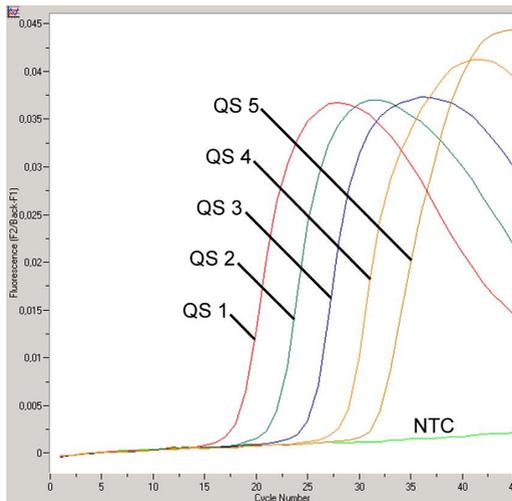


Fig. 6: Detection of the *Quantitation Standards (Parvo B19 LC QS 1 – 5)* in fluorimeter channel F2/Back-F1. NTC: non-template control (negative control).

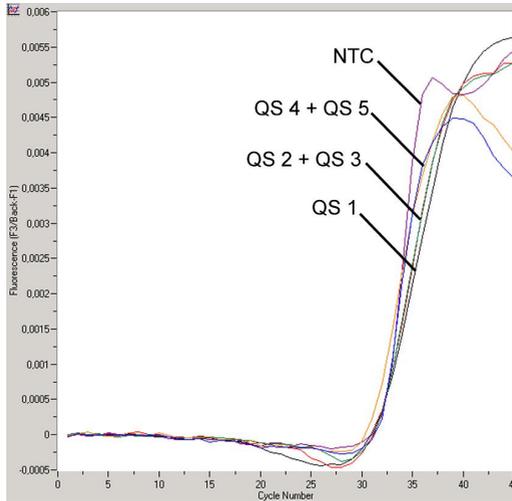


Fig. 7: Detection of the *Internal Control (IC)* in fluorimeter channel F3/Back-F1 with simultaneous amplification of *Quantitation Standards (Parvo B19 LC QS 1 – 5)*. NTC: non-template control (negative control).

11. Troubleshooting

No signal with positive controls (*Parvo B19 LC QS 1 – 5*) in fluorimeter channel F2/Back-F1:

- The selected fluorimeter channel for PCR data analysis does not comply with the protocol.
 - For data analysis select the fluorimeter channel F2/Back-F1 for the analytical parvovirus B19 PCR and the fluorimeter channel F3/Back-F1 for the *Internal Control* PCR.
- Incorrect programming of the temperature profile of the *LightCycler* Instrument.
 - Compare the temperature profile with the protocol (see **section 9.5 Programming of the *LightCycler* 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 LC PCR Kit had expired.
 - Please check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Weak or no signal of the *Internal Control* in fluorimeter channel F3/Back-F1 and simultaneous absence of a signal in channel F2/Back-F1:

- The PCR conditions do not comply with the protocol.
 - Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
- The PCR was inhibited.
 - Make sure that you use a recommended isolation method (see **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 LC PCR Kit had expired.
 - Please check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Signals with the negative controls in fluorimeter channel F2/Back-F1 of the analytical PCR.

- A contamination occurred during preparation of the PCR.
 - Repeat the PCR with new reagents in replicates.
 - If possible, close the PCR tubes directly after addition of the sample to be tested.
 - Strictly pipette the positive controls 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.

12. Specifications

12.1 Analytical Sensitivity

The analytical detection limit as well as the analytical detection limit in consideration of the purification (sensitivity limits) were assessed for the *artus* Parvo B19 LC PCR Kit. The analytical detection limit in consideration of the purification is determined using parvovirus B19-positive clinical specimens in combination with a particular extraction method. In contrast, the analytical detection limit is determined without clinical specimens and independent from the selected extraction method, using a standard of known concentration.

To determine the **analytical sensitivity** of the *artus* Parvo B19 LC PCR Kit, a standard dilution series has been set up from 116.6 to nominal

0.03 Parvo B19 IU*/ μ l and analyzed with the *artus* Parvo B19 LC 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. 8. The analytical detection limit of the *artus* Parvo B19 LC PCR Kit is 1 IU/ μ l ($p = 0.05$). This means that there is a 95 % probability that 1 IU/ μ l will be detected.

Probit analysis: Parvovirus B19 (LightCycler)

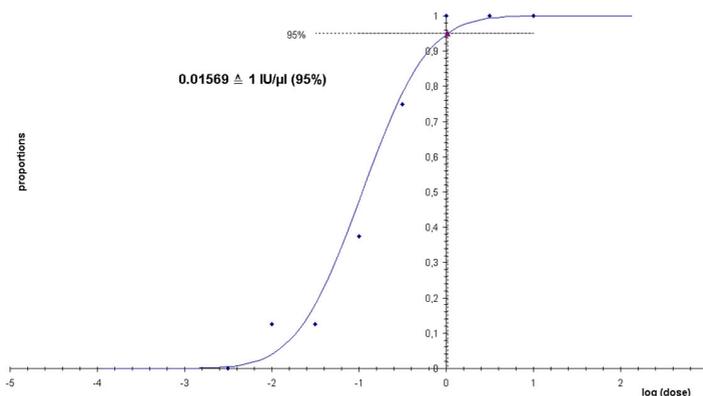


Fig. 8: Analytical sensitivity of the *artus* Parvo B19 LC PCR Kit.

The **analytical sensitivity in consideration of the purification** of the *artus* Parvo B19 LC PCR Kit was determined using a dilution series of the international parvovirus B19 standard (WHO) spiked in clinical plasma specimens. These were subjected to DNA extraction using the QIAamp UltraSens Virus Kit (extraction volume: 1 ml, elution volume: 70 μ l). Each of the six dilutions was analyzed with the *artus* Parvo B19 LC PCR Kit on three different days on eight replicates. The results were determined by a probit analysis. The analytical detection limit in consideration of the purification of the

* The standard is a cloned PCR product, the concentration of which has been calibrated using the international parvovirus B19 standard (WHO).

artus Parvo B19 LC PCR Kit is 125 IU/ml ($p = 0.05$). This means that there is a 95 % probability that 125 IU/ml will be detected.

12.2 Specificity

The specificity of the *artus* Parvo B19 LC 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 30 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 LC Master*.

To determine the specificity of the *artus* Parvo B19 LC 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 (F2/Back-F1)	Internal Control (F3/Back-F1)
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	–	+

12.3 Precision

The precision data of the *artus* Parvo B19 LC 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 LC 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 (see 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 1.62 % (Ct) or 33.37 % (conc.), for the detection of the *Internal Control* 2.13 % (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: <i>Parvo B19 LC QS 5</i>	0.12	0.01	0.36
Intra-assay variability: <i>Internal Control</i>	0.08	0.01	0.27
Inter-assay variability: <i>Parvo B19 LC QS 5</i>	0.43	0.18	1.34
Inter-assay variability: <i>Internal Control</i>	0.66	0.44	2.29
Inter-batch variability: <i>Parvo B19 LC QS 5</i>	0.47	0.22	1.44
Inter-batch variability: <i>Internal Control</i>	0.70	0.44	2.39
Total variance: <i>Parvo B19 LC QS 5</i>	0.52	0.27	1.62
Total variance: <i>Internal Control</i>	0.62	0.39	2.13

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

	Standard Deviation	Variance	Coefficient of Variation [%]
Intra-assay variability: <i>Parvo B19 LC QS 5</i>	0.70	0.49	6.04
Inter-assay variability: <i>Parvo B19 LC QS 5</i>	0.93	0.87	8.01
Inter-batch variability: <i>Parvo B19 LC QS 5</i>	4.12	16.77	52.63
Total variance: <i>Parvo B19 LC QS 5</i>	3.34	11.13	33.37

12.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* Parvo B19 LC PCR Kit. 30 parvovirus B19 negative samples of serum were spiked with 6 IU/μl elution volume of parvovirus B19 control DNA (threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DNA Mini Kit (see **section 9.1 DNA Isolation**) these samples were analyzed with the *artus* Parvo B19 LC 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 LC PCR Kit is ≥ 99 %.

12.5 Reproducibility

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

13. Product Use Limitations

- All reagents may exclusively be used in in vitro diagnostics.
- The kit is intended for in vitro diagnostic use by healthcare professionals.
- 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.

14. Safety Information

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

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

15. Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *artus* Parvo B19 LC PCR Kit has been tested against predetermined specifications to ensure consistent product quality.

16. References

- (1) Hokynar K, Norja P, Laitinen H, Palomäki P, Garbarg-Chenon A, Ranki A, Hedman K, Söderlund-Venermo M. Detection and differentiation of human parvovirus variants by commercial quantitative real-time PCR Tests. *J. Clin. Microbiol.* 2004; 42 (5): 2013 – 2019.
- (2) Mackay IM. Real-time PCR in the microbiology laboratory. *Clin. Microbiol. Infect.* 2004; 10 (3): 190 – 212.
- (3) 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.

17. 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

Mg-Sol

Magnesium Solution

artus Parvo B19 LC PCR Kit

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