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artus® HAdV RG PCR Kit Handbook

Σ 96

Version 1
For use with Rotor-Gene® Q instruments

IVD

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Sample to Insight

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Contents

Intended Use

The *artus*® HAdV RG PCR Kit (96) is an *in-vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of human adenovirus (HAdV) specific DNA.

Summary and Explanation

The *artus* HAdV RG PCR Kit constitutes a ready-to-use system for the detection of HAdV-specific DNA using real-time PCR on Rotor-Gene Q instruments. The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the kit reagents.

Pathogen information

Human adenoviruses (HAdV), first isolated in the 1950s from explanted adenoid tissue, are double-stranded, non-enveloped DNA viruses of the family *Adenoviridae* and belong to the genus *Mastadenovirus*. They have worldwide distribution without a seasonal pattern of infection.

HAdV are classified into 7 species A–G. Species B is further subdivided into B1 and B2. At least 56 different serotypes (HAdV-1 to HAdV-56) have been described to date. Adenoviruses cause a broad range of illnesses including colds, pharyngitis, bronchitis, pneumonia, diarrhea, conjunctivitis (eye infection), fever, cystitis (bladder inflammation or infection), rash illness and neurologic disease.

The symptoms of the disease caused by an adenovirus species depend on the preferred tissue tropism of the virus. For example, respiratory disease is often caused by species B1, C or E, ocular disease by species B, D or E, gastroenteritis is known to be generally induced

by species A, F or G, whereas kidney and urinary tract infections are often associated with HAdV of the species B2.

Epidemiologic characteristics of the adenoviruses vary according to type. While some human adenoviruses are endemic in parts of the world and infection is usually acquired during childhood, other types cause sporadic infection and occasional outbreaks. All HAdV are transmitted by direct contact, fecal-oral transmission and occasionally waterborne transmission.

While the majority of HAdV infections are self-limited, serious pneumonias have occurred sporadically in otherwise healthy persons. Additionally, some types can establish persistent asymptomatic infections in tonsils, adenoids and intestines of infected hosts, and shedding can occur for months or years. Reactivation of latent infections in immunocompromised hosts, such as transplant recipients, can result in a life-threatening disseminated disease.

HAdV are very resistant to different environmental conditions and highly contagious, thus nosocomial outbreaks of adenovirus-associated disease, such as epidemic keratoconjunctivitis, can occur easily if the good infection-control and hygiene practices are not followed carefully. In some countries mandatory reporting at the local level of government is obligatory for some cases of HAdV outbreaks.

Principle of the Procedure

The HAdV RG Master A and HAdV RG Master B contain reagents and enzymes for the specific amplification of target regions within the HAdV genome and for the direct detection of the specific amplicon in the fluorescence channel Cycling Green of Rotor-Gene Q instruments.

In addition, the *artus* HAdV RG PCR Kit contains a heterologous amplification system to identify potential failures during the assay process. This is detected as an Internal Control (IC) in fluorescence channel Cycling Yellow of Rotor-Gene Q instruments.

Probes specific for HAdV DNA are labeled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labeled with the fluorophore JOE™. The use of probes labeled with spectrally distinguishable fluorophores enables simultaneous detection and quantification of HAdV DNA as well as detection of the Internal Control in the corresponding channels of the Rotor-Gene Q instrument.

Materials Provided

Kit contents

***artus* HAdV RG PCR Kit**		**(96)**
Catalog number		**4530265**
Number of reactions		**96**
Blue	HAdV RG Master A	8 x 60 µl
Purple	HAdV RG Master B	8 x 180 µl
Green	HAdV RG IC	1 x 1000 µl
Red	HAdV QS*	4 x 250 µl
White	H_2O	1 x 500 µl
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*The *artus* HAdV RG PCR Kit contains 4 Quantification Standards (QS1–QS4).

Materials Required but Not Provided

Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Reagents

- QIAamp DNA Mini Kit (QIAGEN cat. no. 51304 or 51306; see "DNA extraction", page 11)

Consumables

- 0.1 ml Strip Tubes and Caps, for use with 72-well rotor (QIAGEN, cat. no. 981103 or 981106)

- Nuclease-free, low DNA-binding microcentrifuge tubes for preparing master mixes
- Nuclease-free pipet tips with aerosol barriers

Equipment

- Rotor-Gene Q MDx 5plex, Rotor-Gene Q 5plex or Rotor-Gene Q 6plex instrument
- Rotor-Gene Q software version 2.3.1 or higher
- Loading Block 72 x 0.1 ml Tubes, aluminum block for manual reaction setup (QIAGEN, cat. no. 9018901)
- Dedicated adjustable pipets for sample preparation
- Dedicated adjustable pipets for PCR master mix preparation
- Dedicated adjustable pipets for dispensing template DNA
- Vortex mixer
- Benchtop centrifuge with rotor for 2 ml reaction tubes

Warnings and Precautions

For in vitro diagnostic use.

Read all instructions carefully before using the test.

Warnings

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.

Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free disposable pipet tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for specimen preparation, reaction setup and amplification/detection activities. The workflow in the laboratory should proceed in a unidirectional manner. Always wear disposable gloves in each area, and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separate from all other components of the kit.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

Reagent Storage and Handling

Kit components

The *artus* HAdV RG PCR Kit is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been

compromised during shipment, contact QIAGEN Technical Services for assistance. Upon receipt, store all kit components at –30°C to –15°C.

Avoid thawing and freezing Master reagents (more than two times) as this may reduce assay performance. Freeze the reagents in aliquots if they are to be used intermittently. Do not store reagents at 4°C for longer than 2 hours. Protect HAdV RG Master A and HAdV RG Master B from light.

The *artus* HAdV RG PCR Kit includes:

- Two Master reagents (HAdV RG Master A and HAdV RG Master B)
- Template Internal Control (HAdV RG IC)
- Four Quantification Standards (HAdV QS1–4)
- PCR-grade water (H_2O)

HAdV RG Master A and HAdV RG Master B reagents contain all components (buffer, enzymes, primers and probes) for amplification and detection of HAdV-specific DNA and the Internal Control in a single reaction.

The Quantification Standards contain standardized concentrations of HAdV-specific DNA. These can be used individually as positive controls or together to generate a standard curve, which can be used to determine the concentration of HAdV-specific DNA in the sample. The concentrations of the Quantification Standards are shown in Table 1.

Table 1. Concentration of Quantification Standards

Quantification Standard	Concentration (copies/µl)
QS1	10,000
QS2	1000
QS3	100
QS4	10

Procedure

DNA extraction

HAdV-specific target sequences are amplified from DNA. As assay performance is dependent on the quality of the template DNA, make sure to use a sample preparation kit that yields DNA suitable for use in downstream PCR.

The QIAamp DNA Mini Kit (QIAGEN, cat. no. 51304 or 51306) is recommended for DNA purification for use with the *artus* HAdV RG PCR Kit. Carry out DNA purification according to the instructions in *QIAamp DNA Mini Handbook*.

As the wash buffers in the QIAamp DNA Mini Kit contain ethanol, carry out an additional centrifugation step prior to elution. Place the QIAamp Mini spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge for 10 minutes at approximately 17,000 x *g* (~13,000 rpm) in a benchtop centrifuge.

Important: The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

Important: Ethanol is a strong inhibitor in real-time PCR. If your sample preparation kit uses wash buffers containing ethanol, make sure to remove all traces of ethanol prior to elution of the nucleic acid.

Internal Control

The *artus* HAdV RG PCR Kit contains a heterologous Internal Control, which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

If the Internal Control is used as a PCR inhibition control, but not as a control for the sample preparation procedure, add the Internal Control directly to the mixture of HAdV RG Master A and HAdV RG Master B, as described in step 2b of the protocol (page 14).

Regardless of which method/system is used for nucleic acid extraction, the Internal Control must not be added directly to the specimen. The Internal Control should always be added to the specimen/lysis buffer mixture. The volume of Internal Control to be added to the specimen/lysis buffer mixture depends only on the elution volume, and represents 10% of the elution volume. For example, when using the QIAamp DNA Mini Kit, the DNA is eluted in 60 µl Buffer AE. Therefore, add 6 µl Internal Control to the specimen/lysis buffer mixture of each sample.

Important: Do not add the Internal Control and the carrier RNA directly to the specimen.

Protocol: Detection of HAdV-specific DNA

Important points before starting

- Before beginning the procedure, read "Precautions", page 8.
- Take time to familiarize yourself with the Rotor-Gene Q instrument before starting the protocol. See the instrument user manual.
- Make sure that at least one positive control and one negative control (PCR-grade water) are included per PCR run.

Things to do before starting

- Make sure that the cooling block (accessory of the Rotor-Gene Q instrument) is precooled to 2–8°C.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing) and centrifuged briefly.

Procedure

1. Place the desired number of PCR tubes into the adapters of the cooling block.
2. If you are using the Internal Control to monitor the DNA isolation procedure and to check for possible PCR inhibition, follow step 2a. If you are using the Internal Control exclusively to check for PCR inhibition, follow step 2b.

   Use the Internal Control according to step 2b for all samples, controls and Quantification Standards to be analyzed.

   2a. The Internal Control has already been added to the isolation (see "Internal Control", page 12). In this case, prepare a master mix according to Table 2.

   The reaction mix typically contains all of the components needed for PCR, except the sample.

Table 2. Preparation of master mix (Internal Control used to monitor DNA isolation and check for PCR inhibition)

Component	1 reaction	12 reactions
HAdV RG Master A	5 µl	60 µl
HAdV RG Master B	15 µl	180 µl
Total volume	20 µl	240 µl

2b. The Internal Control must be added directly to the mixture of HAdV RG Master A and HAdV Master B. In this case, prepare a master mix according to Table 3.

The reaction mix contains all of the components needed for PCR, except the sample.

Table 3. Preparation of master mix (Internal Control used exclusively to check for PCR inhibition)

Component	1 reaction	12 reactions
HAdV RG Master A	5 µl	60 µl
HAdV RG Master B	15 µl	180 µl
HAdV RG IC	1 µl	12 µl
Total volume	21 µl	252 µl

* 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.

3. Pipet 20 µl of the master mix into each PCR tube. Then add 10 µl of the eluted sample DNA and mix well by pipetting repeatedly up and down. Correspondingly, add 10 µl of a positive control or Quantification Standard or 10 µl water (PCR-grade water) as a negative control.

   Make sure to have at least one positive control and one negative control per run. For quantification, use all 4 Quantification Standards (QS1–QS4).

4. Close the PCR tubes. Make sure that the locking ring (accessory of the Rotor-Gene Q instrument) is placed on top of the rotor.

5. For the detection of HAdV-specific DNA, create a temperature profile according to the following steps.

Setting the general assay parameters	**Figures 1, 2, 3, 4**
Initial activation of the hot-start enzyme	**Figure 5**
Amplification of the DNA	**Figure 6**
Adjusting the fluorescence channel sensitivity	**Figure 7**
Starting the run	**Figure 8**

All specifications refer to the Rotor-Gene Q software version 2.3.1, and higher. Please find further information on programming Rotor-Gene Q instruments in the instrument user manual. In the illustrations these settings are framed in bold black.

6. First, open the **New Run Wizard** dialog box with the **Advanced** version and select **Two Step** (Figure 1). Click **Next** to continue.

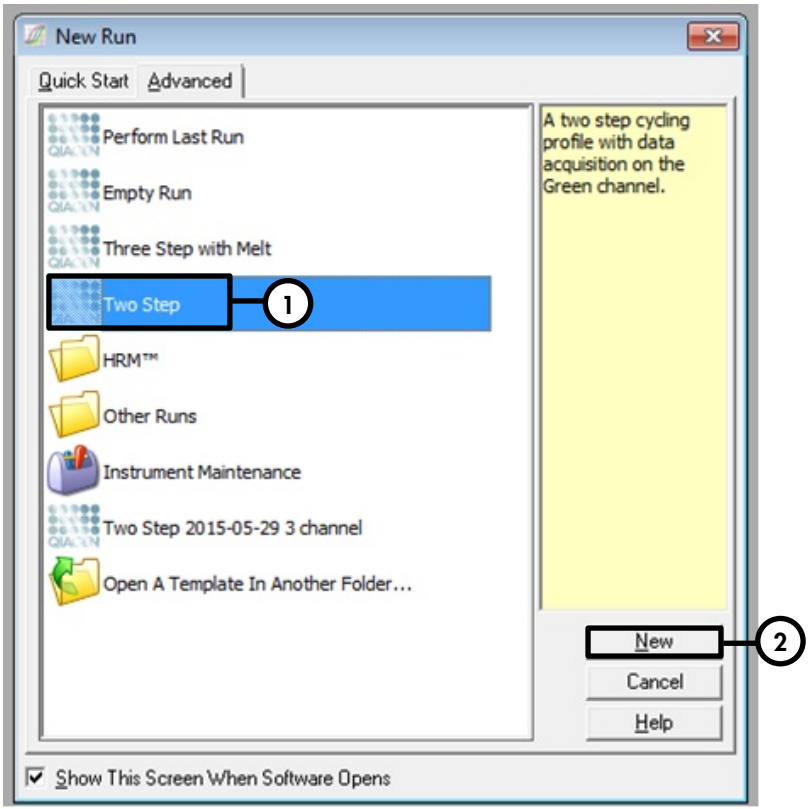

Figure 1. The New Run dialog box.

7. In the next **New Run Wizard** dialog box (Figure 2), check the **Locking Ring Attached** box and click **Next**.

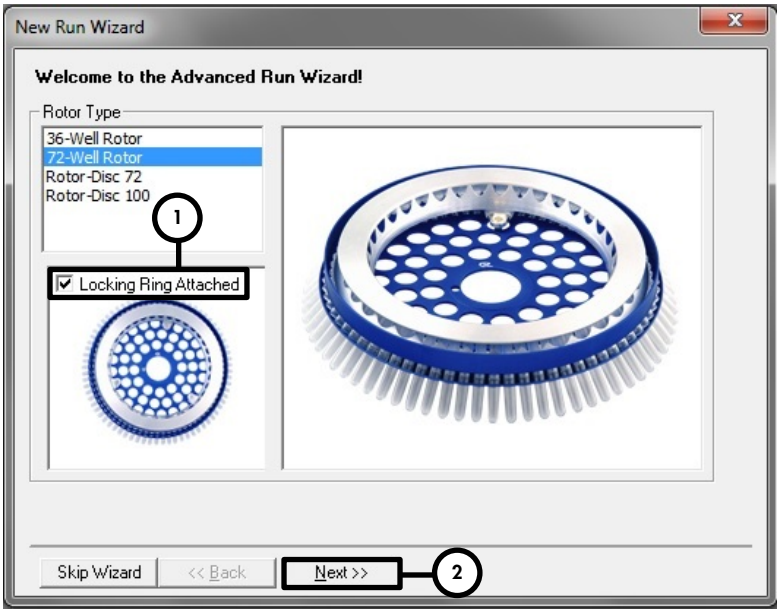

Figure 2. The New Run Wizard dialog box.

8. Select **30** for the PCR reaction volume and click **Next** (Figure 3).

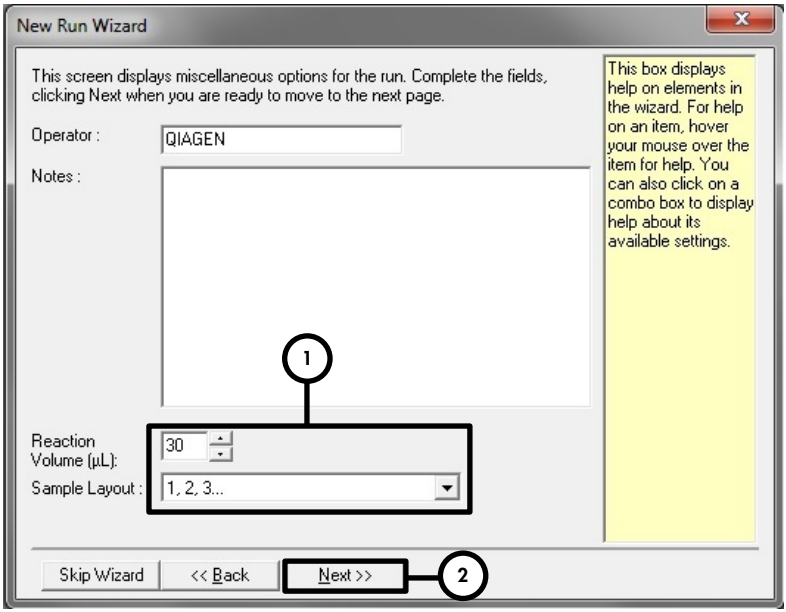

Figure 3. Setting the general assay parameters.

9. Click the **Edit Profile** button in the next **New Run Wizard** dialog box (Figure 4), and program the temperature profile as shown in Figures 5–6.

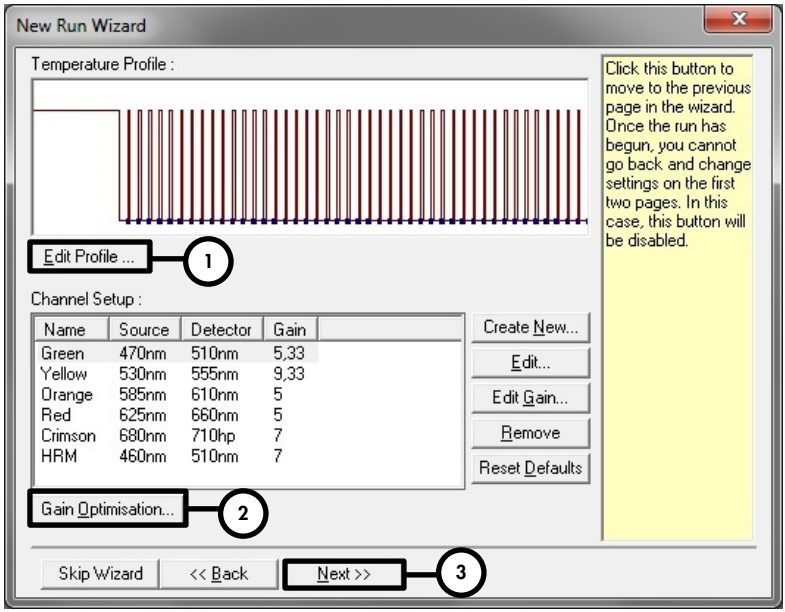

Figure 4. Editing the profile.

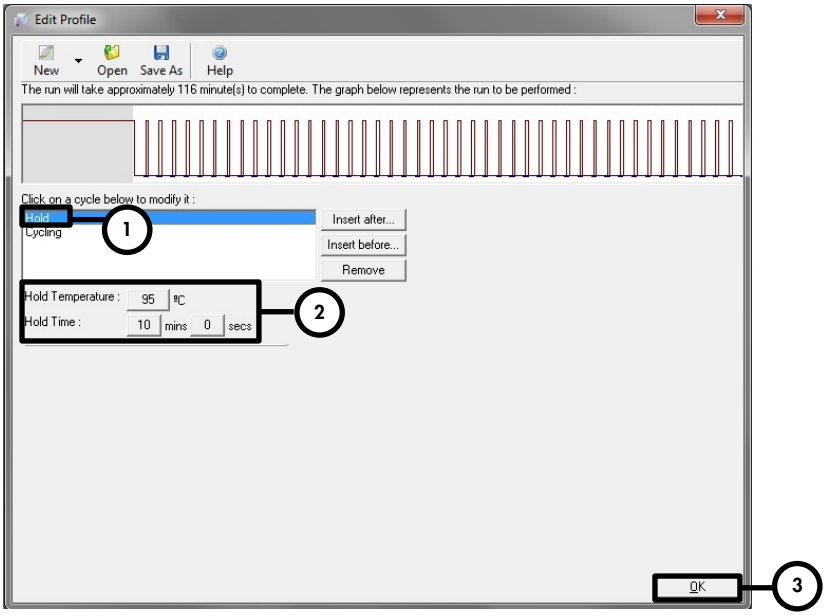

Figure 5. Initial activation of the hot-start enzyme.

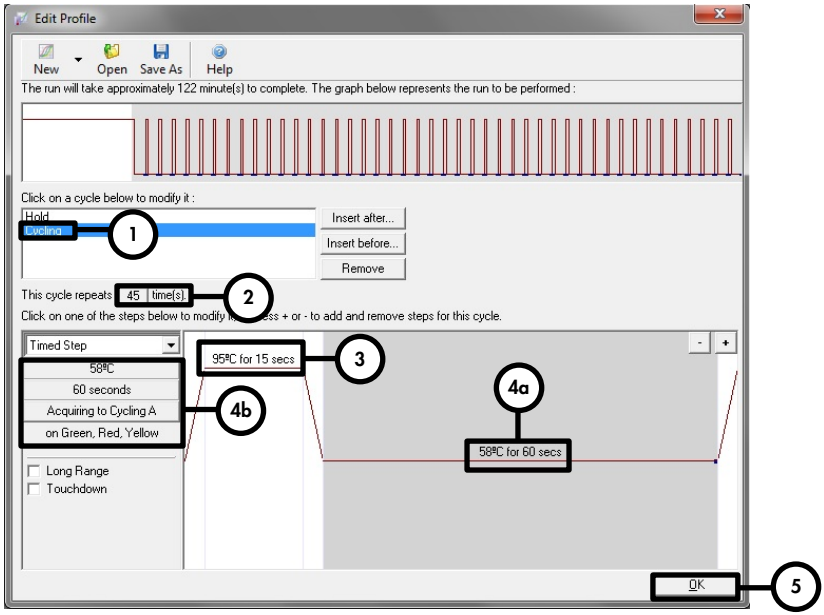

Figure 6. Amplification of the DNA.

10. The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click **Gain Optimisation** in the **New Run Wizard** dialog box (see Figure 4, Step 2) to open the **Auto-Gain Optimisation Setup** dialog box (Figure 7). Check the **Perform Optimisation Before 1st Acquisition Box** (Figure 7). Make sure that both channels (Green and Yellow) are selected for **Auto-Gain Optimisation** (Figure 7). (Find channels in the drop down menu under **Channel Settings** and click **Add**.) Click **Close** of the **Auto-Gain Optimisation Setup** dialog box when the gain calibration is completed.

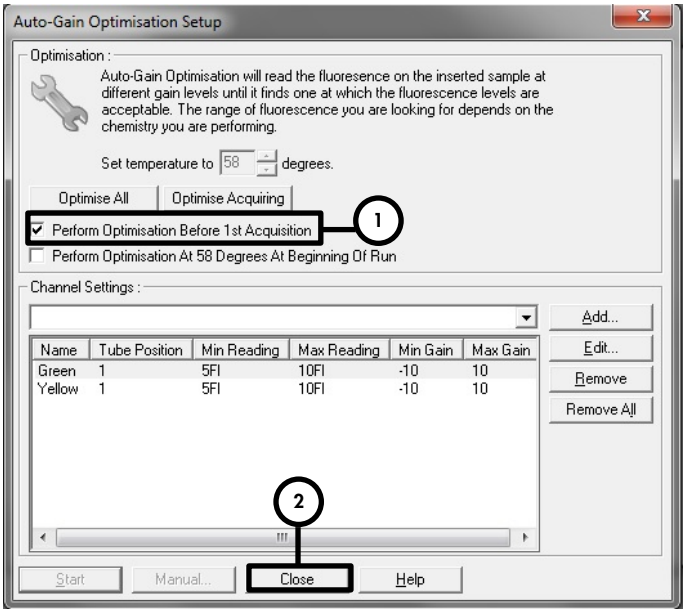

Figure 7. Adjusting the fluorescence channel sensitivity.

11. The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (Figure 8). Click **Start Run**.

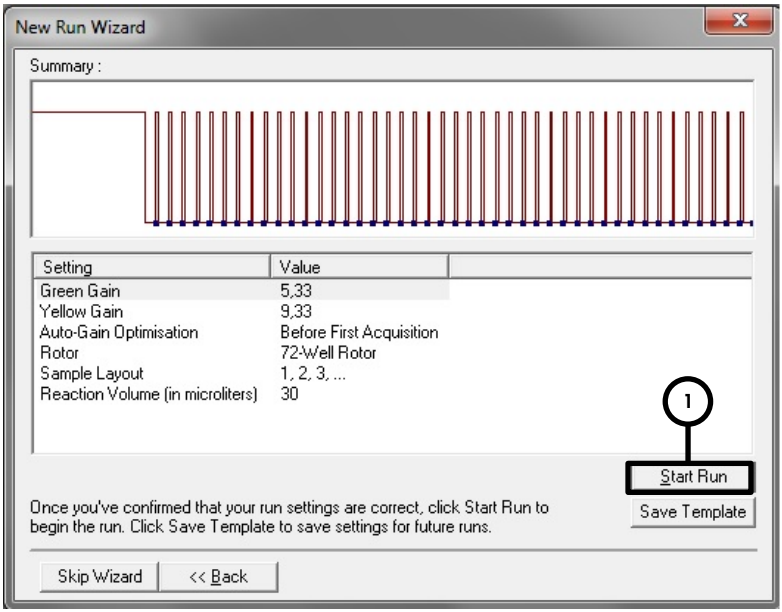

Figure 8. Starting the run.

12. After the run is finished, analyze the data (see "Interpretation of Results", page 24).

Interpretation of Results

Run validity

Valid qualitative run

The following control conditions must be met for a qualitative run to be valid (Table 4).

Table 4. Control conditions for a valid qualitative run

	Detection channel	
Control ID	**Cycling Green**	**Cycling Yellow**
Positive control (QS)	POSITIVE	POSITIVE
Negative control	NEGATIVE	POSITIVE

Invalid qualitative run

A qualitative run is invalid if the run has not been completed or if any of the control conditions for a valid qualitative run have not been met.

In case of an invalid qualitative run, repeat the PCR or extract DNA from the original samples again if no DNA is left over.

Valid quantitative run

A quantitative run is valid if all control conditions for a valid qualitative run have been met (see Table 4, above). Furthermore, for accurate quantification results, a valid standard curve needs to be generated. For a valid quantitative run, the standard curve must have the following control parameter values (Table 5).

Table 5. Control parameters for a valid standard curve

Control parameter	Valid value
Slope	–3.743/–2.765
PCR efficiency	85%/130%
R squared (R^2)	>0.98

Invalid quantitative run

A quantitative run is invalid if the run has not been completed or if any of the control conditions for a valid quantitative run have not been met.

In case of an invalid quantitative run, repeat the PCR or extract DNA from the original samples again if no DNA is left over.

Qualitative analysis

A summary of results interpretation is shown in Table 6.

Table 6. Summary of results interpretation

	Detection channel		
Sample ID	**Cycling Green**	**Cycling Yellow**	**Result interpretation**
A	POSITIVE	POSITIVE*	HAdV-specific DNA detected.
B	NEGATIVE	POSITIVE	HAdV-specific DNA not detected. Sample does not contain detectable amounts of HAdV-specific DNA.
C	NEGATIVE	NEGATIVE	PCR inhibition or reagent failure. Repeat procedure using original sample or collect and test a new sample.

* Detection of the Internal Control in the Cycling Yellow detection channel is not required for positive results in the Cycling Green detection channel. A high HAdV load in the sample can lead to reduced or absent Internal Control signals.

Quantitative analysis

The *artus* HAdV RG PCR Kit contains 4 Quantification Standards (QS). To generate a standard curve for quantitative analysis, these have to be defined as standards with appropriate concentrations (see Table 1, page 11). A standard curve for quantitative analysis can be generated using standards of known concentrations.

$C_T = m \log(N_0) + b$

- C_T = Threshold cycle
- m = Slope
- N_0 = Initial concentration
- b = Intercept

The concentrations of positive samples of unknown concentration can be derived from the standard curve (Figure 9).

$N_0 = 10^{(C_T-b)/m}$

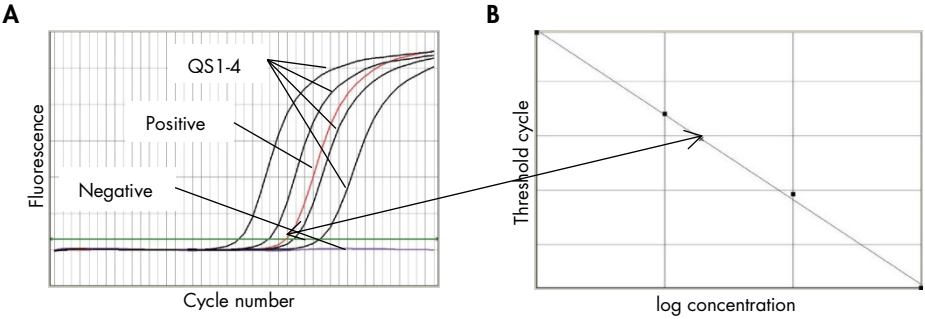

Figure 9. Quantification Standards, a positive and a negative sample displayed in (A) an amplification plot and (B) standard curve analysis.

Note: The concentration of the sample is displayed in copies/µl and refers to the concentration of viral DNA in the eluate.

Use the following formula to determine the viral load of the original sample.

$$\text{Viral load (sample) [copies/ml]} = \frac{\text{Volume (eluate) [µl] x viral load (eluate) [copies/µl]}}{\text{Sample input [ml]}}$$

Limitations

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- Take extreme care to preserve the purity of the components of the kit and reaction setups. Closely monitor all reagents for impurities and contamination. Discard any reagents suspected of contamination.

- Appropriate specimen collection, transport, storage and processing procedures are required for optimal performance of this assay.
- Do not use this assay directly on the specimen. Perform the applicable nucleic acid extraction procedures prior to using this assay.
- The presence of PCR inhibitors may cause false-negative or invalid results.
- Potential mutations within the target regions of the HAdV genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- As with any diagnostic test, interpret the results obtained using the *artus* HAdV RG PCR Kit in consideration of all clinical and laboratory findings.

Quality Control

Each lot of *artus* HAdV RG PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Performance Characteristics

As there is no international standard available for adenovirus, quantitative performance evaluation of the *artus* HAdV RG PCR Kit was carried out using genomic DNA from a characterized HAdV3 isolate (species B).

For qualitative performance evaluation, genomic DNA of adenovirus species A–F was analyzed using the *artus* HAdV RG PCR Kit. Genomic DNA was obtained from ATCC® (American Type Culture Collection) and from characterized cell culture isolates. For the analysis of species G (serotype HAdV-52), a plasmid was used containing the according target sequence (Table 7).

Table 7. Adenovirus species and serotypes analyzed with the *artus* HAdV RG PCR Kit

HAdV species	HAdV serotype	Source	Result with the *artus* HAdV RG PCR Kit
Species A	HAdV-12	ATCC-VR-863D	Positive
	HAdV-31	Characterized isolate from cell culture	Positive
	HAdV-18	Plasmid	Positive
Species B1	HAdV-3	ATCC-VR-3, ATCC-VR-857D	Positive
	HAdV-7	Plasmid	Positive
Species B2	HAdV-35	ATCC-VR-718D	Positive
	HAdV-11	Characterized isolate from cell culture	Positive
	HAdV-55	Plasmid	Positive
Species C	HAdV-1	ATCC-VR-1	Positive
	HAdV-2	Plasmid	Positive
	HAdV-5	ATCC-VR-5D	Positive
	HAdV-6	Characterized isolate from cell culture	Positive
Species D	HAdV-37	ATCC-VR-929D	Positive
	HAdV-19	Plasmid	Positive
Species E	HAdV-4	ATCC-VR-1572D	Positive
Species F	HAdV-41	ATCC-VR-930D	Positive
Species G	HAdV-52	Plasmid	Positive

Analytical sensitivity

The analytical sensitivity of the *artus* HAdV RG PCR Kit is defined as the concentration (copies per µl of the eluate) of HAdV-specific DNA that can be detected with a positivity rate of ≥95%. The analytical sensitivity was determined by analysis of a dilution series of quantified genomic adenovirus DNA (group B, subtype 3) (Table 8).

Table 8. PCR results used to calculate the analytical sensitivity of the *artus* HAdV RG PCR Kit

Input concentration (copies/µl)	Number of replicates	Number of positives	Hit rate (%)	Internal Control
31.6	18	18	100	Valid
10.0	18	18	100	Valid
3.2	18	18	100	Valid
1.0	18	18	100	Valid
0.3	18	12	67	Valid
0.1	18	7	39	Valid
0.03	18	3	17	Valid
0.01	18	1	6	Valid
0.003	18	0	0	Valid

The analytical sensitivity of the *artus* HAdV RG PCR Kit, determined by probit analysis, for detection of HAdV-specific DNA is 1.07 copies/µl (95% confidence interval [CI]: 0.58–2.99 copies/µl).

Analytical specificity

The analytical specificity of the *artus* HAdV RG PCR Kit is ensured by careful selection of the oligonucleotides (primers and probes). The oligonucleotides are checked by sequence comparison analysis against publically available sequences to ensure that all relevant adenovirus genotypes are detected.

The analytical specificity of the *artus* HAdV RG PCR Kit was evaluated by testing a panel of genomic DNA/RNA extracted from other pathogens causing similar symptoms as adenovirus infections and by testing human genomic DNA (Table 9).

Table 9. Organisms tested to demonstrate the analytical specificity of the *artus* HAdV RG PCR Kit

	Detection channel	
Organism	Cycling Green (HAdV)	Cycling Yellow (IC)
Human genomic DNA	Negative	Valid
Varicella-zoster virus	Negative	Valid
Herpes simplex virus 1	Negative	Valid
Herpes simplex virus 2	Negative	Valid
Epstein-Barr virus	Negative	Valid
Human herpesvirus 6 (A, B)	Negative	Valid
Human herpesvirus 7	Negative	Valid
Cytomegalovirus	Negative	Valid
BK virus	Negative	Valid
JC virus	Negative	Valid
Simian virus 40	Negative	Valid
Hepatitis A virus	Negative	Valid
Hepatitis B virus	Negative	Valid
Hepatitis C virus	Negative	Valid
Human immunodeficiency virus 1	Negative	Valid
Parvovirus B19	Negative	Valid
Escherichia coli (EHEC)	Negative	Valid
Pseudomonas aeruginosa	Negative	Valid
Chlamydia pneumoniae	Negative	Valid
Mycoplasma pneumoniae	Negative	Valid
Neisseria meningitidis	Negative	Valid
Streptococcus pyogenes	Negative	Valid

Organism	Detection channel	
	Cycling Green (HAdV)	Cycling Yellow (IC)
Haemophilus influenzae	Negative	Valid
Coronavirus	Negative	Valid
Influenza virus A (incl. H1N1-2009), B	Negative	Valid
Respiratory syncytial virus A, B	Negative	Valid
Parainfluenzavirus 1–4	Negative	Valid
Human metapneumovirus	Negative	Valid
Rhinovirus	Negative	Valid

The *artus* HAdV RG PCR Kit did not cross-react with any of the specified organisms.

Linear range

The linear range of the *artus* HAdV RG PCR Kit was evaluated by analyzing a logarithmic dilution series of quantified genomic HAdV-2 DNA (species C) using concentrations ranging from 1 x 10^9 to 0.1 copies/µl. Six replicates per dilution were analyzed.

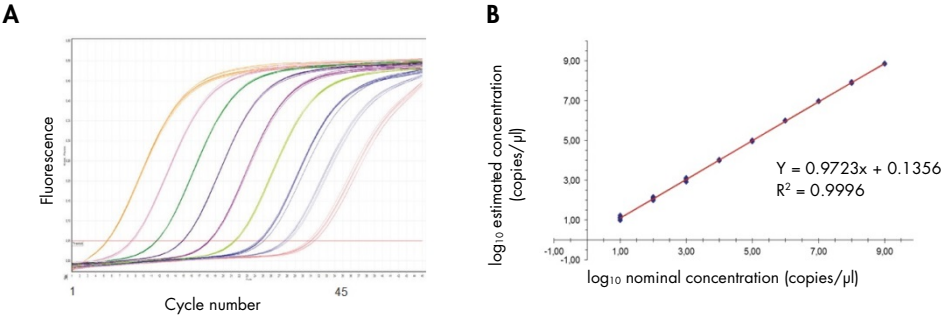

Figure 10. Amplification curve (A) and linear regression analysis (B) of a dilution series of genomic DNA from HAdV-2 (species C).

The linear range of the *artus* HAdV RG PCR Kit extends over an interval of at least 8 orders of magnitude for HAdV-specific DNA.

Precision

The precision of the *artus* HAdV RG PCR Kit was determined by intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots).

Variability data are expressed in terms of standard deviation and coefficient of variation. The data are based on quantification analysis of defined concentrations of genomic HAdV DNA and on threshold cycle (C_T) values in terms of the Internal Control (Tables 10 and 11, respectively). At least 6 replicates per sample were analyzed for intra-assay, inter-assay and inter-lot variability. Total variance was calculated by combining the 3 analyses.

Table 10. Precision data for the HAdV-DNA specific detection system of the *artus* HAdV RG PCR Kit

HAdV-specific system	Average conc. (copies/µl)	Standard deviation	Coefficient of variation (%)
Intra-assay variability	26.88	4.87	18.13
Inter-assay variability	35.11	8.65	24.63
Inter-lot variability	27.39	4.65	16.97
Total variance	32.37	8.44	26.09

Table 11. Precision data for the Internal Control of the *artus* HAdV RG PCR Kit

Internal Control	Average threshold cycle (C_T)	Standard deviation	Coefficient of variation (%)
Intra-assay variability	21.97	0.15	0.67
Inter-assay variability	22.12	0.19	0.87
Inter-lot variability	22.05	0.25	1.12
Total variance	22.02	0.22	0.99

Diagnostic evaluation

The diagnostic sensitivity and specificity of the *artus* HAdV RG PCR Kit are regularly evaluated by analyzing reference and diagnostic samples previously analyzed with reference methods (i.e., in-house PCR, DFA, shell vial culture, electron microscopy, Luminex® technology). So far, 223 specimens derived from smears, nasopharyngeal aspirates, bronchial secretions, stool samples, urine samples, plasma or eye smears collected in different laboratories were tested for determining the diagnostic sensitivity and specificity of the *artus* HAdV RG PCR Kit. Out of these 223 specimens, 50 were predicated to be HAdV positive and 173 were predicated to be HAdV negative by reference methods (Table 12). Four samples tested positive for HAdV (C_T values 35.2, 36.8, 40.0 and 37.9) with the *artus* HAdV RG PCR Kit that previously tested negative with an in-house PCR test. All 50 specimens predicted to contain HAdV DNA were confirmed as HAdV positive by analysis with the *artus* HAdV RG PCR Kit.

Table 12. Diagnostic evaluation of the *artus* HAdV-6 RG PCR Kit

		***artus* HAdV RG PCR Kit**	
		NEGATIVE	**POSITIVE**
Reference method	**NEGATIVE**	169	4*
	POSITIVE	0	50

* C_T values: 35.2, 36.8, 40.0 and 37.9.

Repeatability

Specificity, sensitivity and accuracy of quantification of the *artus* HAdV RG PCR Kit were evaluated by analyzing established proficiency panels for adenovirus. To ensure repeatability of the *artus* HAdV RG PCR Kit, specificity and sensitivity are evaluated by analyzing established proficiency panels for adenovirus as well as characterized diagnostic samples on a regular basis.

Table 13. Results of the analysis of a proficiency panel for HAdV (QCMD) using the *artus* HAdV RG PCR Kit

Proficiency panel			*artus* HAdV RG PCR Kit	
Sample ID	Sample content	Expected conc. (copies/ml)	Result	Internal Control
14-01	HAdV-1	2793	Positive	Valid
14-02	HAdV-1	13,213	Positive	Valid
14-03	HAdV-1	2793	Positive	Valid
14-04	HAdV-1	4093	Positive	Valid
14-05	HAdV-4	2032	Positive	Valid
14-06	HAdV-4	21,281	Positive	Valid
14-07	Negative	0	Negative	Valid
14-08	HAdV-14	426,580	Positive	Valid
14-09	HAdV-5	241	Positive	Valid
14-10	HAdV-5	1820	Positive	Valid

Symbols

The symbols in the following table are used in these instructions for use.

Symbol	Symbol definition
	Contains sufficient for 96 tests
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Temperature limitation
	Manufacturer

Symbol	Symbol definition
	Use by
	Material number
	Global Trade Item Number
	Consult instructions for use

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
artus HAdV RG PCR Kit (96)	For 96 reactions: Master A, Master B, 4 Quantification Standards, Internal Control, H_2O (PCR grade water)	4530265
QIAamp DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA Mini Kit (250)	For 250 DNA preps: 250 QIAamp Mini Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51306
Rotor-Gene Q and accessories		
Rotor-Gene Q MDx 5plex System	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002023
Rotor-Gene Q MDx 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002022

Product	Contents	Cat. no.
Rotor-Gene Q 5plex Priority Package Plus	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes Priority Package with software, installation, training, 3-year warranty on parts and labor, and 3 preventive maintenance visits	9001866
Rotor-Gene Q 5plex Priority Package	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes Priority Package with software, installation, training, 2-year warranty on parts and labor, and 2 preventive maintenance visits	9001865
Rotor-Gene Q 5plex System	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9001640
Rotor-Gene Q 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9001570

Product	Contents	Cat. no.
Rotor-Gene Q 6plex Priority Package Plus	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes Priority Package with software, installation, training, 3-year warranty on parts and labor, and 3 preventive maintenance visits	9001870
Rotor-Gene Q 6plex Priority Package	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes Priority Package with software, installation, training, 2-year warranty on parts and labor, and 2 preventive maintenance visits	9001869
Rotor-Gene Q 6plex System	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9001660
Rotor-Gene Q 6plex Platform	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9001590

Product	Contents	Cat. no.
Loading Block 72 x 0.1 ml Tubes	Aluminum block for manual reaction setup with a single-channel pipet in 72 x 0.1 ml tubes	9018901
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 ml (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106

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