altona DIAGNOSTICS

Instructions for Use

RealStar®
Adenovirus PCR Kit 1.0

07/2018 EN

RealStar®

RealStar®

Adenovirus PCR Kit 1.0

For use with

m2000rt (Abbott Diagnostics)

Mx 3005P™ QPCR System (Stratagene)

VERSANT® kPCR Molecular System AD (Siemens Healthcare)

ABI Prism® 7500 SDS (Applied Biosystems)

Rotor-Gene® 6000 (Corbett Research)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

CFX96™ Real-Time PCR Detection System (Bio-Rad)

LightCycler® 480 Instrument II (Roche)

CE

IVD

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Content

1. Intended Use

The RealStar® Adenovirus PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of human adenovirus (HAdV) specific DNA.

2. Kit Components

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Blue	Master A	8	60
Purple	Master B	8	180
Green	Internal Control	1	1000
Red	QS1-4*	4	250
White	Water (PCR grade)	1	500

* The RealStar® Adenovirus PCR Kit 1.0 contains Quantification Standards (QS) at four different concentrations (see Chapter 6. Product Description)

3. Storage

- The RealStar® Adenovirus PCR Kit 1.0 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 altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 8.1 Sample Preparation)
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

NOTE

It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).

5. Background 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 a worldwide distribution without 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. All HAdV are transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission.

Human 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 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 infections of kidney and urinary tract are often associated with species B2.

Epidemiologic characteristics of the adenoviruses vary by 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. Some HAdV 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.

6. Product Description

The RealStar® Adenovirus PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of human adenovirus (HAdV) specific DNA.

Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

Probes specific for HAdV DNA are labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of HAdV specific DNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of two processes in a single tube assay:

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® Adenovirus PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Four Quantification Standards (QS1 - QS4)
- PCR grade water

Master A and Master B contain all components (PCR buffer, DNA polymerase, magnesium salt, primers and probes) to allow PCR mediated amplification and detection of HAdV specific DNA and Internal Control in one reaction setup.

The Quantification Standards contain standardised concentrations of HAdV specific DNA. The Quantification Standards 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 Quantification Standards have the following concentrations:

Quantification Standard	Concentration [copies/µl]
QS1	1.00E+04
QS2	1.00E+03
QS3	1.00E+02
QS4	1.00E+01

6.1 Real-Time PCR Instruments

The RealStar® Adenovirus PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- m2000rt (Abbott Diagnostics)
- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

6.2 Sample Types

The following sample types have been validated with the RealStar® Adenovirus PCR Kit 1.0:

- Human EDTA plasma

If an appropriate nucleic acid extraction procedure is applied additional sample types can be used along with the RealStar® Adenovirus PCR Kit 1.0. The suitability of the nucleic acid extraction procedure has to be validated by the user.

7. Warnings and Precautions

Read the Instructions for Use carefully before using the product.

- Before first use check the product and its components for:
  - Integrity
  - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
  - Correct labelling
  - Frozenness upon arrival
- 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 specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.

- Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- 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 separated 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 autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Procedure

8.1 Sample Preparation

Extracted DNA is the starting material for the RealStar® Adenovirus PCR Kit 1.0.

The quality of the extracted DNA has a profound impact on the performance of the entire test system. It is recommended to ensure that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® DNA Mini Kit (QIAGEN)
- QIAsymphony® (QIAGEN)
- NucliSENS® easyMag® (bioMérieux)

- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with RealStar® Adenovirus PCR Kit 1.0 has to be validated by the user.

If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

CAUTION

If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

CAUTION

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

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® Adenovirus PCR Kit 1.0 contains a heterologous Internal Control (IC), 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 IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**21 µl**	**252 µl**

- If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, add the IC during the nucleic acid extraction procedure.
- No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.
- If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

CAUTION

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.

CAUTION

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

8.3 Reaction Setup

- ► Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Quantification Standard, Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

- ► Make sure that at least one Positive (QS) and one Negative Control is used per run.
- ► For quantification purposes all Quantification Standards (QS1 to QS4) should be used.
- ► Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.
- ► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® Adenovirus PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

► Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
HAdV specific DNA	HAdV	FAM™	(None)
Internal Control (IC)	IC	JOE™	(None)

9.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Denaturation	Hold	1	-	95	10:00
Amplification	Cycling	45	-	95	00:15
			yes	58	01:00

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® Adenovirus PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **valid**, if the following control conditions are met:

Control ID	Detection Channel	
	FAM™	JOE™
Positive Control (QS)	+	+/-*
Negative Control	-	+

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.1.3 Valid Diagnostic Test Run (quantitative)

A **quantitative** diagnostic test run is **valid**, if all control conditions for a **valid qualitative** diagnostic test run are met [see chapter 10.1.1 Valid Diagnostic Test Run (qualitative)]. The **quantification** results are **valid** if the generated **standard curve** reaches the following control parameter value:

Control Parameter	Valid Value
R square (R^2)	≥ 0.98

NOTE

Not all real-time PCR instruments display the R square (R^2) value. For detailed information, please refer to the user manual of the respective instrument.

10.1.4 Invalid Diagnostic Test Run (quantitative)

A **quantitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid quantitative** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Detection Channel		Result Interpretation
FAM™	JOE™	
+	+*	HAdV specific DNA detected.
-	+	No HAdV specific DNA detected. Sample does not contain detectable amounts of HAdV specific DNA.
-	-	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high HAdV DNA load in the sample can lead to a reduced or absent Internal Control signal.

10.2.2 Quantitative Analysis

The RealStar® Adenovirus PCR Kit 1.0 includes four Quantification Standards (QS). In order to generate a **standard curve** for quantitative analysis, these have to be defined as **standards** with appropriate concentrations (see chapter 6. Product Description). Using **standards** of known concentrations a standard curve for quantitative analysis can be generated.

$$C_t = m \cdot \log (N_0) + b$$

C_t = Threshold Cycle
m = Slope
N_0 = Initial Concentration
b = Intercept

Derived from the standard curve positive samples of unknown concentrations can be quantified.

$$(C_t - b) / m$$

$$\mathbf{N_0 = 10}$$

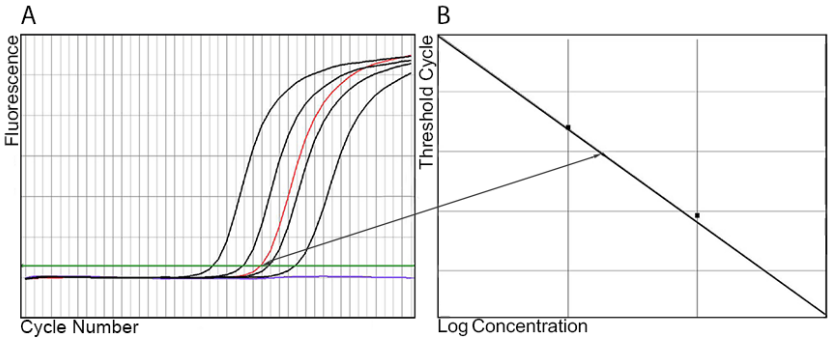


Figure 1: Quantification Standards (black), a positive (red) and a negative sample (blue) displayed in the Amplification Plot [**A**] and Standard Curve analysis [**B**]

NOTE

The concentration of the "Sample" is displayed in copies/µl and refers to the concentration in the eluate.

To determine the **viral load of the original sample**, the following formula has to be applied:

$$\text{Viral load (}\mathbf{Sample}\text{) [copies/ml]} = \frac{\text{Volume (}\mathbf{Eluate}\text{) [µl]} \cdot \text{Viral load (}\mathbf{Eluate}\text{) [copies/µl]}}{\text{Sample Input [ml]}}$$

11. Performance Evaluation

Since there is no international standard available for adenovirus, quantitative performance evaluation of the RealStar® Adenovirus PCR Kit 1.0 was done by using genomic DNA of a characterized HAdV-2 isolate (species C) that was calibrated by a photometrically quantified plasmid containing the target sequence of HAdV-2 (species C).

For qualitative performance evaluation, genomic DNA of adenovirus species A-F was analysed using the RealStar® Adenovirus PCR Kit 1.0. Genomic DNA was obtained from ATCC (American Type Culture Collection), NIBSC (National Institute for Biological Standards and Control) 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 1: Adenovirus species and serotypes analysed with the RealStar®Adenovirus PCR Kit 1.0

HAdV species	HAdV serotype	Source	Results with the RealStar® Adenovirus PCR Kit 1.0
A	HAdV-12	ATCC-VR-863D	Positive
A	HAdV-31	characterzied isolate from cell culture	Positive
A	HAdV-18	plasmid	Positive
B1	HAdV-3	ATCC-VR-3, ATCC-VR-857D, characterized isolate from cell culture	Positive
B1	HAdV-7	plasmid	Positive
B2	HAdV-35	ATCC-VR-718D	Positive
B2	HAdV-11	characterized isolate from cell culture	Positive
B2	HAdV-55	plasmid	Positive
C	HAdV-1	ATCC-VR-1, characterized isolate from cell culture	Positive
C	HAdV-2	CE Marked Material Human Adenovirus serotype 2 for Nucleic Acid Amplification, characterized isolate from cell culture, plasmid	Positive
C	HAdV-5	ATCC-VR-5D, characterized isolate from cell culture	Positive
C	HAdV-6	characterized isolate from cell culture	Positive
D	HAdV-37	ATCC-VR-929D, characterized isolate from cell culture	Positive
D	HAdV-19	plasmid	Positive
E	HAdV-4	ATCC-VR-1572, ATCC-VR-1572D, characterized isolate from cell culture	Positive
F	HAdV-41	ATCC-VR-930D	Positive
G	HAdV-52	plasmid	Positive

Additionally, the adenovirus serotypes HAdV-1 (species C), HAdV-4 (species E), HAdV-34 (species B) and HAdV-41 (species F) as part of the proficiency panels QCMD2010 (Qualtity Control for Molecular Diagnostics) were detected using the RealStar® Adenovirus PCR Kit 1.0.

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® Adenovirus PCR Kit 1.0 is defined as the concentration (copies/µl of the eluate) of HAdV specific DNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series ofgenomic HAdV-2 DNA (species C).

Table 2: PCR results used for the calculation of the analytical sensitivity with respect to the detection of HAdV specific DNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
10.100	16	16	100
3.200	16	16	100
1.010	16	15	94
0.320	16	11	69
0.101	16	4	25
0.032	16	1	6
0.010	16	0	0
0.003	16	0	0
0.001	16	0	0

The analytical sensitivity of the RealStar® Adenovirus PCR Kit 1.0 was determined by Probit analysis:

- For the detection of adenovirus specific DNA, the analytical sensitivity is 1.09 copies/µl [95% confidence interval (CI): 0.62 - 3.08 copies/µl]

11.2 Analytical Specificity

Cross Reactivity

The analytical specificity of the RealStar® Adenovirus PCR Kit 1.0 was evaluated by testing a panel of genomic DNA/RNA extracted from other pathogens causing similar symptoms to adenovirus infections and by testing human genomic DNA.

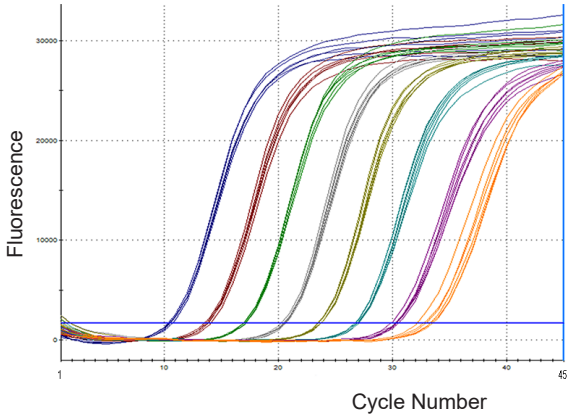
The RealStar® Adenovirus PCR Kit 1.0 did not cross-react with any of the following pathogens:

- BK virus
- Cytomegalovirus
- Epstein-Barr virus
- Hepatitis A virus
- Hepatitis B virus
- Hepatitis C virus
- Herpes simplex virus 1
- Herpes simplex virus 2
- Human herpesvirus 6A
- Human herpesvirus 6B
- Human herpesvirus 7
- Human herpesvirus 8
- Human immunodeficiency virus 1
- Human metapneumovirus A
- Human metapneumovirus B
- Human parainfluenza virus 1
- Human parainfluenza virus 2
- Human parainfluenza virus 3
- Human parainfluenza virus 4a/b
- Human parvovirus B19
- Human respiratory syncytial virus A
- Human respiratory syncytial virus B
- Influenza A virus
- Influenza B virus
- JC virus
- Rhinovirus 16
- Simian virus 40
- Varicella-zoster virus
- *Chlamydophila pneumoniae*
- *Escherichia coli*
- *Haemophilus influenzae*
- *Mycoplasma pneumoniae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Streptococcus pyogenes*

11.3 Linear Range

The linear range of the RealStar® Adenovirus PCR Kit 1.0 was evaluated by analysing a logarithmic dilution series of quantified genomic HAdV-2 DNA (species C) using concentrations ranging from 4.00E+07 to 4.00E+00 copies/µl. Each dilution was analysed in six replicates.

A



B

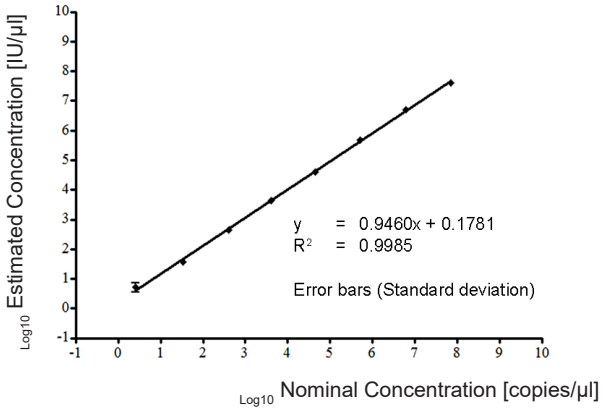


Figure 2: Amplification curves [**A**] and linear regression [**B**] of an analysed dilution series of HAdV specific DNA

The linear range of the RealStar® Adenovirus PCR Kit 1.0 extends over an interval of at least **seven** orders of magnitude.

11.4 Precision

Precision of the RealStar® Adenovirus PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the three analyses.

Variability data are expressed in terms of standard deviation and coefficient of variation. The data are based on quantification analysis of defined concentrations of HAdV specific DNA and on threshold cycle (C_t) value in terms of the Internal Control. At least six replicates per sample were analysed for intra-assay, inter-assay and inter-lot variability.

Table 3: Precision data for the detection of HAdV specific DNA

HAdV	Average Conc. [copies/µl]	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	473.43	30.44	6.43
Inter-Assay Variability	450.13	38.93	8.65
Inter-Lot Variability	463.55	34.98	7.55
Total Variability	451.31	37.86	8.39

Table 4: Precision data for the detection of the Internal Control

Internal Control	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	24.07	0.15	0.63
Inter-Assay Variability	24.13	0.18	0.77
Inter-Lot Variability	24.40	0.41	1.68
Total Variability	24.30	0.34	1.40

11.5 Diagnostic Evaluation

The diagnostic sensitivity and specificity of the RealStar® Adenovirus PCR Kit 1.0 is evaluated regularly by analysing reference samples and diagnostic samples previously tested with a reference method (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 RealStar® Adenovirus PCR Kit 1.0.

Out of these 223 specimens, 50 were predicated to be HAdV positive and 173 were predicated to be HAdV negative by reference methods. Four samples were tested

HAdV positive (C_t -values 35.2, 36.8, 40.0, 37.9) with the RealStar® Adenovirus PCR Kit 1.0 that were 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 RealStar® Adenovirus PCR Kit 1.0.

Table 5: Results of the evaluation of the diagnostic sensitivity and specificity of the RealStar® Adenovirus PCR Kit 1.0

		RealStar® Adenovirus PCR Kit 1.0	
		-	+
Reference method	-	169	4*
	+	0	50

* C_t-values 35.2, 36.8, 40.0, 37.9

12. Limitations

- Strict compliance with the Instructions for Use is required for optimal results.
- 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. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.

- The presence of PCR inhibitors (e.g. heparin) may cause underquantification, 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 underquantification and/or failure to detect the presence of the pathogens.
- As with any diagnostic test, results of the RealStar® Adenovirus PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the altona Diagnostics GmbH ISO EN 13485-certified Quality Management System, each lot of RealStar® Adenovirus PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

15. Literature

Versalovic, James, Carroll, Karen C.,Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); ATCC® (American Type Culture Collection); CFX96™ (Bio-Rad); Cy® (GE Healthcare); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); SmartCycler® (Cepheid); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gene®, QIAamp®, MinElute®, QIAsymphony® (QIAGEN); VERSANT® (Siemens Healthcare).

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The RealStar® Adenovirus PCR Kit 1.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

17. Explanation of Symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Catalogue number
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade identification number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Note
	Version

Notes:

Notes:

Notes:

always a drop ahead.

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