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RealStar®
Adenovirus PCR Kit 1.0

11/2012

RealStar®

Adenovirus PCR Kit 1.0

For use with

m2000rt (Abbott Diagnostics)

Mx 3005P™ QPCR System (Stratagene)

VERSANT™ kPCR Molecular System AD (Siemens)

ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)

LightCycler® 480 Instrument II (Roche)

Rotor-Gene™ 3000/6000 (Corbett Research)

Rotor-Gene Q5/6 plex Platform (QIAGEN)

CFX96™/Dx Real-Time System (BIO-RAD)

CE

IVD For *in vitro* diagnostic use

REF Product No.: 301013

Σ 96 rxns

Store at -25°C ... -15°C

November 2012

altona Diagnostics GmbH • Mörkenstraße 12 • 22767 Hamburg

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

*The RealStar® Adenovirus PCR Kit 1.0 contains four Quantification Standards (QS1-QS4).

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 at -20°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 at +4°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 (chapter 6. Product Description)
- Appropriate nucleic acid extraction system or kit
- 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 instruments have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.***

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. 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 infections of kidneys and urinary tract is often associated with HAdV of the 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. 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.

RealStar® Adenovirus PCR Kit 1.0

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. The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

The test is based on real-time PCR technology, utilizing 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 the 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 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)
- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene™ 3000/6000 (Corbett Research)
- Rotor-Gene Q 5/6 plex Platform (QIAGEN)
- CFX96™/Dx Real-Time System (BIO-RAD)

The RealStar® Adenovirus PCR Kit 1.0 consists of:

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

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

The Quantification Standards contain standardized 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 in the sample.

The following concentrations are used:

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

7. Warnings and 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 if 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 pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) specimen preparation, (ii) reaction setup and (iii) amplification/detection activities. Workflow in the laboratory should proceed in 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 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 use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

RealStar® Adenovirus PCR Kit 1.0

8. Instructions for Use

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 has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

The following nucleic acid extraction systems and kits are recommended:

- KingFisher® Flex (Thermo Scientific) with ExtraStar® Purification Kit (altona Diagnostics)*
- VERSANT™ Molecular System SP (Siemens)
- HighPure® Viral Nucleic Acid Kit (Roche)
- QIAamp® DNA Mini Kit (QIAGEN)

* for *stool* and *swab* samples.

If using a spin column based sample preparation procedure including washing buffers containing ethanol, 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 is highly recommended.

NOTE

> ⚠ ***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***
>
> ⚠ ***Ethanol is a strong inhibitor in real-time PCR. If your sample preparation system is using washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support:

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

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, the Master Mix is set up 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, the IC has to be added 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 depends always and only 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.

NOTE

⚠ ***Never add the Internal Control directly to the specimen!***

- If the IC was added during the sample preparation procedure, the Master Mix is set up 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**

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).
- Make sure that at least one Positive and one Negative Control are 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 up and down pipetting.
- Close the 96-well reaction plate with an appropriate 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).

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

9. Programming the Real-Time PCR Instruments

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

9.1 Settings

- Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX

9.2 Fluorescent Detectors (Dyes)

- Define the fluorescent detectors (dyes):

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

9.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Denaturation	Hold	1	-	95 °C	10:00 min
Amplification	Cycling	45	-	95 °C	0:15 min
			√	58 °C	1:00 min

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the manual of the respective instrument.
For detailed instructions regarding data analysis of the RealStar® Adenovirus PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support.

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run (qualitative)

For a **valid** diagnostic test run (**qualitative**), the following control conditions must be met:

Control ID	FAM Detection Channel	JOE Detection Channel
Positive Control (QS)	POSITIVE	POSITIVE
Negative Control	NEGATIVE	POSITIVE

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)

For the validity of a **quantitative** diagnostic test run, all control conditions of a **valid qualitative** diagnostic test run must be met [chapter 10.1.1 Valid Diagnostic Test Run (qualitative)]. Furthermore, for accurate quantification results a valid **standard curve** has to be generated. For a **valid quantitative** diagnostic test run, the following control parameter values of the standard curve should be achieved:

Control Parameter	Valid Value
Slope	- 3.00 / - 3.74
PCR Efficiency	85 % / 115 %
R square (R^2)	> 0.98

NOTE

> ⚠ ***Not all parameters are displayed by the software of the different real-time PCR instruments. For detailed information, please refer to the 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** 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

Sample ID	FAM Detection Channel	JOE Detection Channel	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 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. High HAdV load in the sample can lead to reduced or absent Internal Control signals.

10.2.2 Quantitative Analysis

The RealStar® Adenovirus PCR Kit 1.0 provides four Quantification Standards (QS). In order to generate a **standard curve** for quantitative analysis, these have to be defined as **standards** with the appropriate concentrations (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_o = Initial Concentration
b = Intercept

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

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

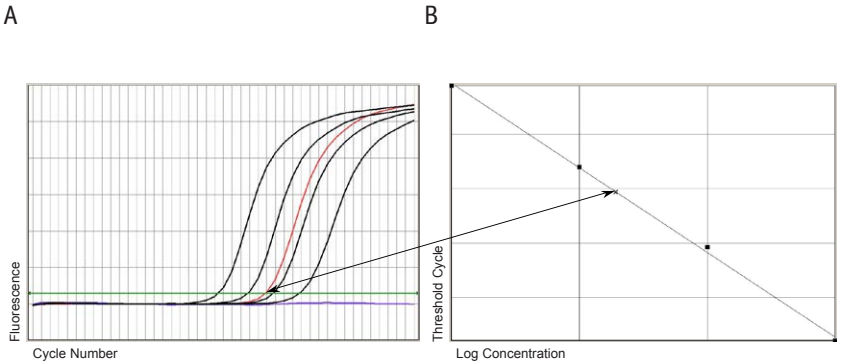

Figure 1: Quantification Standards (black), a positive sample (red) and a negative sample (blue) displayed in the Amplification Plot (**A**) and Standard Curve analysis (**B**).

NOTE

> ⚠ ***The concentration of your "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:

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

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.

Table1: 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
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, characterized isolate from cell culture	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, characterized isolate from cell culture	Positive
	HAdV-2	CE Marked Material Human Adenovirus serotype 2 for Nucleic Acid Amplification, characterized isolate from cell culture, plasmid	Positive
	HAdV-5	ATCC-VR-5D, characterized isolate from cell culture	Positive
	HAdV-6	characterized isolate from cell culture	Positive
Species D	HAdV-37	ATCC-VR-929D, characterized isolate from cell culture	Positive
	HAdV-19	plasmid	Positive
Species E	HAdV-4	ATCC-VR-1572, ATCC-VR-1572D, characterized isolate from cell culture	Positive
Species F	HAdV-41	ATCC-VR-930D	Positive
Species 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 and QCMD2011 (Quality 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 per µl of the eluate) of HAdV DNA molecules that can be detected with a positivity rate of ≥ 95%. The analytical sensitivity was determined by analysis of dilution series of quantified genomic HAdV-2 DNA (species C).

Table 2: PCR results used for the calculation of the analytical sensitivity of the RealStar® Adenovirus PCR Kit 1.0

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]	Internal Control
10.10	16	16	100	Valid
3.200	16	16	100	Valid
1.010	16	15	94	Valid
0.320	16	11	69	Valid
0.101	16	4	25	Valid
0.032	16	1	6	Valid
0.010	16	0	0	Valid
0.003	16	0	0	Valid
0.001	16	0	0	Valid

Analytical sensitivity of the RealStar® Adenovirus PCR Kit 1.0 determined by Probit analysis is 1.09 copies/µl [95% confidence interval (CI): 0.62 - 3.08 copies/µl].

11.2 Analytical Specificity

The analytical specificity of the RealStar® Adenovirus PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against public available sequences to ensure that all relevant Adenovirus genotypes are detected.
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.

Table 3: Organisms tested to demonstrate the analytical specificity of the RealStar® Adenovirus PCR Kit 1.0

	RealStar® Adenovirus PCR Kit 1.0	
Organisms	**FAM Channel (HAdV)**	**JOE Channel (Internal Control)**
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
Human Herpesvirus 8	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

Continuation of table 3

Organisms	FAM Channel (HAdV)	JOE Channel Internal Control
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
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 RealStar® Adenovirus PCR Kit 1.0 did not cross-react with any of the specified organisms.

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.

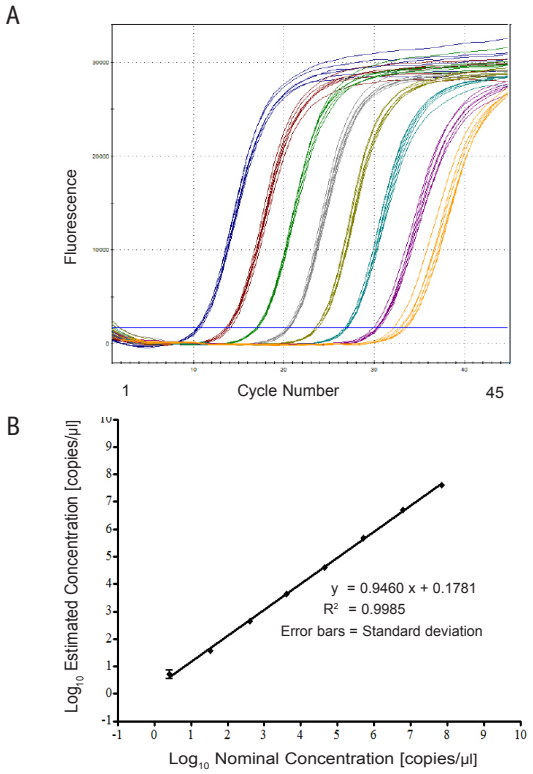

Figure 2: Amplification curves [A] and linear regression [B] of an analysed dilution series of genomic DNA from HAdV-2 (species C).

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

11.4 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 4: Results of the evaluation of the diagnostic sensitivity and specificity of the RealStar® Adenovirus PCR Kit 1.0.

		RealStar® Adenovirus PCR Kit 1.0	
		NEGATIVE	POSITIVE
Reference Method	NEGATIVE	169	4*
	POSITIVE	0	50

* C_t values 35.2, 36.8, 40.0, 37.9

11.5 Precision

Precision data of the RealStar® Adenovirus PCR Kit 1.0 were 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).
Variability data are expressed in terms of standard deviation, variance and coefficient of variation. The data are based on quantification analysis of defined concentrations of genomic HAdV 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. Total variance was calculated by combining the three analyses.

Table 5: Precision data for HAdV-specific DNA of the RealStar® Adenovirus PCR Kit 1.0 (concentration in copies/µl)

HAdV specific System	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 Variance	451.31	37.86	8.39

Table 6: Precision data for the Internal Control of the RealStar® Adenovirus PCR Kit 1.0 (C_t-values)

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 Variance	24.30	0.34	1.40

11.6 Repeatability

Specificity, sensitivity and accuracy of quantification of the RealStar® Adenovirus PCR Kit 1.0 were evaluated by analysing established proficiency panels for Adenovirus. To ensure repeatability of the RealStar® Adenovirus PCR Kit 1.0, specificity and sensitivity are evaluated by analysing established proficiency panels for Adenovirus as well as characterized diagnostic samples on a regular basis.

Table 7: Results of the RealStar® Adenovirus PCR Kit 1.0 analysing a proficiency panel for HAdV (QCMD)

Proficiency Panel			RealStar® HAdV PCR Kit 1.0	
Sample ID	Sample Content	Expected Conc. [copies/ml]	Result	Internal Control
11-01	HAdV-34	993	Positive	Valid
11-02	HAdV-1	1358	Positive	Valid
11-03	HAdV-1	272	Positive	Valid
11-04	Negative	-	Negative	Valid
11-05	HAdV-1	10765	Positive	Valid
11-06	HAdV-4	13804	Positive	Valid
11-07	HAdV-34	7413	Positive	Valid
11-08	HAdV-1	1250	Positive	Valid

12. Limitations and 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.
- 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 is not to 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 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 pathogen.
- As with any diagnostic test, results of the RealStar® Adenovirus PCR Kit 1.0 should 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. Trademarks and Disclaimers

RealStar®, ExtraStar® (altona Diagnostics GmbH); Mx 3005P™ (Stratagene); ABI Prism® (Applied Biosystems); HighPure®, LightCycler® (Roche); Rotor-Gene™, QIAamp® (QIAGEN); VERSANT™ (Siemens); KingFisher® (Thermo Scientific); CFX96™ (BIO-RAD).

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

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

Not available in all countries.

16. Explanation of Symbols

In vitro diagnostic medical device

Product number

Batch code


Contains sufficient for “n” tests/reactions (rxns)


Temperature limitation


Version


Use until


Caution


Consult instructions for use


Manufacturer