MBPCR272 Hi-PCR® Adenovirus Probe PCR Kit

Description

Human adenoviruses (HAdVs) belong to the Adenoviridae family, which are nonenveloped double-stranded DNA viruses. As human pathogens, HAdVs are responsible for a wide spectrum of diseases in the respiratory, ocular, gastrointestinal, and renal tracts, commonly. Although many adenovirus infections are subclinical, these viruses may cause acute respiratory disease (types of 1-7, 14 and 21); conjunctivitis (types 3, 7, 8, 11, 14, 19 and 37); acute hemorrhagic cystitis (11 and 21); acute respiratory disease (ARD) (types 3, 4, 7, 14 and 21); and gastroenteritis (types 31, 40, 41 and 52). Adenoviruses gain access to susceptible individuals through the mouth, the nasopharynx, or the conjunctiva. Although initial infection may occur via the respiratory route, fecal oral transmission accounts for most adenovirus infections in young children because of the prolonged shedding of viruses in feces. The enteric adenoviruses 40 and 41 have been recognized as the second most important etiological agents of viral gastroenteritis in children. These viruses, in contrast to other adenoviruses, are not shed in respiratory secretions; thus, their transmission is limited to the oral fecal route. The traditional method to type these pathogens was based on virus neutralization and hemagglutination assays, which are both time-consuming and difficult. HiMedia has developed a Real-time PCR assay method for detection of human adenoviruses. HAdV strains were used for development of a qualitative real-time PCR assay using primers targeting a conserved region of all human adenoviruses with a specific TaqMan probe.

NOTE: Hi-PCR® Adenovirus Probe PCR Kit is for *in-vitro* use only.

Intended Use

Hi-PCR® Adenovirus Probe PCR Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The kit is recommended for sensitive and specific detection of adenovirus in clinical samples.

Product Description

Hi-PCR® Adenovirus Probe PCR Kit includes primer-probe sets specific to detect DNA from human adenoviruses. In addition, an internal control (IC) is used for testing successful reactions. The kit also provides positive controls for validity of the test.

Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to ensure proper and intended functioning of all the reagents and is recommended to be used in every run to assess optimal performance.

Negative Template Control

A Negative template control is needed to ensure that the reagents, equipment, and environment used in the assay is not contaminated with adenovirus DNA. In this reaction, Nuclease free water is used as the template. It is recommended to have a minimum of one reaction of negative control per run.

ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

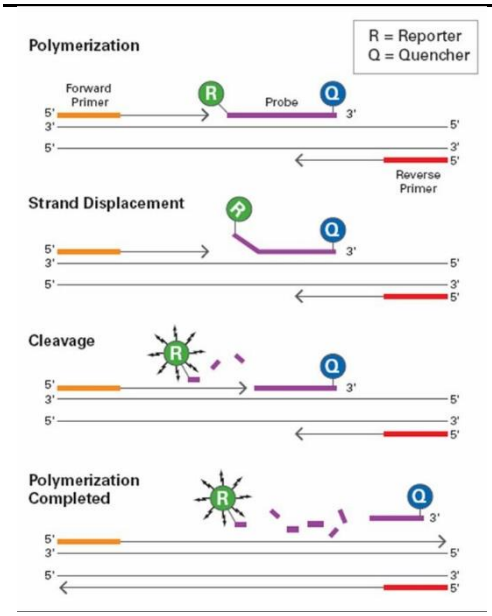
Internal Control

This is a control sequence that should amplify in all clinical samples which indicates the presence of sufficient DNA from human gene indicating the specimen is of acceptable quality. An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

Principle

Real-Time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a target DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. Hi-PCR® Adenovirus Probe PCR Kit is designed to detect a conserved region of all human adenoviruses in FAM channel. The internal control is in JOE channel.

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-Time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement: When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle,the DNA polymerae cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'- nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

- Fast and Simple – Real-Time PCR within 1.5 hours
- Highly sensitive and specific for detection of adenoviruses
- Includes all reagents and controls
- Positive controls provided for validity of the test
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q 96® Plus and Insta Q96®AG Real Time PCR Systems

Sample Type: DNA extracted from **nasopharyngeal and oropharyngeal s**wabs

Specimen collection and Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with other body fluids. Safety guidelines may be referred to in individual safety data sheets.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C to -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on the pack.

Kit Contents: The provided PCR kit contains:

Components	Product Code	Reagents provided for (reactions)*		
		25R	50R	100R
Adenovirus Master Mix	DS1569	270 µl	540 µl	1060 µl
Adenovirus Primer-Probe Mix	DS1570	27 µl	54 µl	106µl
Adenovirus Positive Control	DS1571	49 µl	97.2 µl	191 µl
Water	DS0440	49 µl	97.2 µl	191 µl

***For a 20 µL PCR reaction**

Materials needed but not provided: All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q48® M4: Real time PCR System, 48 well block, 4 channels	LA1023
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Automated nucleic acid extraction system and materials	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag16, Insta NX® Mag16Plus	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32Plus	LA1096, MBLA019
Insta NX® Mag96	LA1097
Extraction Kit	
HiPurA® Viral DNA/ RNA Purification Kit	MB582
HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification for Insta NX® Mag96	MB582MPF-96
HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification for Insta NX® Mag32	MB582MPF-32
HiPurA® Pre-filled Cartridges for Viral Nucleic Acid Purification	MB582PC16
Tubes, plates, and other consumables	
Varivol II Micropipette-10 (Capacity: 0.5 to 10 µl)	LA611
Varivol II Micropipette-100 (Capacity: 10 to 100 µl)	LA615
Varivol II Micropipette-1000 (Capacity: 200 to 1000 µl)	LA614
Barrier Tips, Maximum capacity 10 µl	LA749/LA749A
Barrier Tips, Maximum capacity 200 µl	LA751/LA751A
Barrier Tips, Maximum capacity 1000 µl	LA859/LA859A
Micro Centrifuge Tube - B	PW146

Micro Centrifuge Tube-C	PW147
8-strip tubes & optically clear flat caps for PCR	PR17
PCR Tubes, 0.2 mL; PCR Plates	PW1255/ PR2/PR3/PR19
Polypropylene Sealing film	PR21
Optical Sealing film	PR18
RNase Kil™	ML162

Kit Compatibility with Real-Time PCR systems:
Hi-PCR® Adenovirus Probe PCR Kit contains fluorophores compatible to:

Real-Time PCR system	Company	Dye 1	Dye 2
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/ Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	JOE
BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch	Bio-Rad Laboratories, Inc.	FAM	JOE/HEX
QuantStudio™ 5 / Quant Studio™ 6 and 7 Flex Real-Time PCR Systems / QuantStudio™ Dx	Applied Biosystems	FAM	JOE/HEX/VIC
ABI® Prism SDS 7500	Applied Biosystems	FAM	JOE/HEX/VIC
QIAquant 96 & 384 5plex	QIAGEN	FAM	JOE/HEX
Rotor-Gene®6000 & Q	QIAGEN	Green	Yellow
LightCycler® 96	Roche	FAM	JOE/HEX/VIC
LightCycler® 480	Roche	FAM	JOE/HEX/VIC
qTOWER^3	Analytik Jena	FAM	JOE/HEX/VIC

Note: Ensure that the Real-Time PCR system is calibrated for dyes mentioned above and is maintained according to the manufactureŕs instructions and recommendations.

Warning and Precautions
Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred to in the safety data sheets of the product.

Limitations
Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

General Preparation Instructions
- Before use, all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

A. Protocol for PCR Master Mix Preparation

1. In the "Master mix Preparation" area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for several seconds. Keep on ice for later use.
2. Based on the number of specimens to be tested (N), including the PTC and NTC, calculate the volume of the components to be added as N* volume of 1X

Components	Volume to be added for 1X (For a 20 µL reaction)
Adenovirus Master Mix	10.0 µL
Adenovirus Primer-Probe Mix	1.0 µL
Test – Extracted Sample DNA	9.0 µL
Total volume	**20 µL**

3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the reaction system. After all the reagents are added, mix them thoroughly and centrifuge for several seconds.
4. Load 11 µL of master mixture into the 0.1/0.2 mL PCR reaction plate/strips, compatible to the instrument to be used.
5. Add 9 µL of nuclease free water to the negative control tube.
6. In the "Nucleic acid handling" area, add 9 µL of Adenovirus Positive Control and extracted test DNA into the respective tubes.
7. Tightly cap the strips or seal the plate using an optically clear adhesive film.
8. Briefly, spin the strips/tubes to settle the reagent to the bottom of the tube.
9. Place the plate/strips in the Real-time PCR machine and set the PCR program.

B. Recommended PCR program:

1.	Initial denaturation	:	95°C for 10 minutes	Nos. od cycles: 01
2.	Denaturation	:	95°C for 15 seconds	Nos. of cycles: 45
3.	Annealing	:	60°C for 45 seconds (Sampling/Plate Read)	
	Detection Channel	:	FAM/JOE	

Note: Please select the passive reference dye and quencher as "NONE".

C. Data Analysis

When the following controls meet the stated requirement the PCR run is considered valid, and the specimens can be considered for interpretation.

Control	Target	
	Adeno (FAM)	IC (JOE)
Positive Template Control (PTC)	**+**	**+**
Negative Template Control (NTC)	**-**	**-**

All clinical samples should exhibit IC amplification at or below 35 Ct value, thus suggesting the presence of sufficient DNA from human gene indicating the specimen is of acceptable quality. If the samples are from non-human origin (animal species) or if the DNA is extracted from cell culture supernatant sample, usually such samples exhibit low or no amplification for IC gene.

D. Data Interpretation

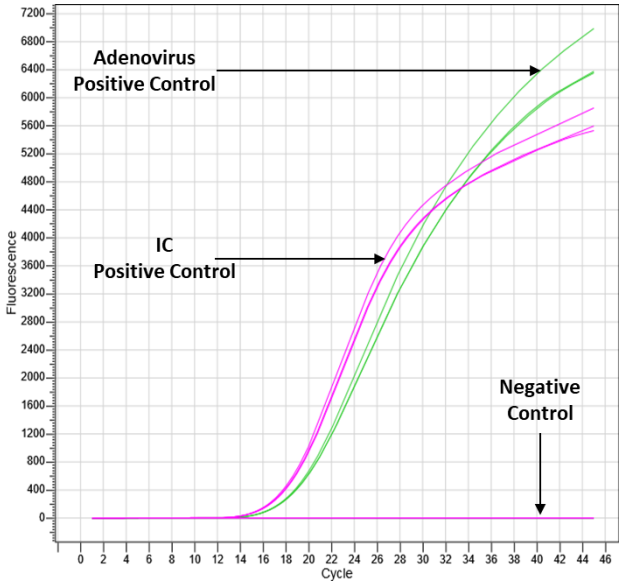
Targets		Assay Interpretation
Adeno (FAM)	IC (JOE)	
+	+	Adenovirus Positive
-	+	Adenovirus Negative

+	-	IC can be inhibited if the sample load is very high in the same.
-	-	Invalid test. Repeat extraction or obtain a new specimen for analysis

Note

- Positive results are indicative of the presence of adenovirus DNA. However, clinical correlation along with patient history is necessary to determine patient infection status.

Amplification Data



Sr. No	Target	Ct value	
		PTC	NTC
1	Adenovirus	19.63	--
2	IC	18.40	--

Note: Image representing probe based Real-Time amplification of Adenovirus and IC (Ct values provided in table are for representation).

Performance Evaluation

Inclusivity - Analytical Sensitivity

In-silico analysis for the assessment of inclusivity for the Hi-PCR® Adenovirus Probe PCR Kit was conducted by mapping the primers and probes against all the available sequences of adenoviruses in NCBI GenBank. The Hi-PCR® Adenovirus Probe PCR Kit targets 100% of the known strains of human adenoviruses.

Cross-reactivity - Analytical Specificity

Wet testing analysis was performed against the recommended list of organisms for respiratory pathogens. No cross-reaction was observed with any strains mentioned below.

SARS-CoV-2	*Staphylococcus aureus*
Human Coronavirus HKU1 RNA	*Chlamydophila pneumoniae*
Human Rhinovirus 16 strain 11757	*Mycoplasma pneumoniae*
Human Metapneumovirus hMPV RNA	*Legionella pneumoniae*
Enterovirus D68 strain Fermon	*Mycobacterium tuberculosis*
Human Parainfluenza 1	Measles
Human Parainfluenza 2	*Aspergillus Niger*
Human Parainfluenza 3	Influenza A

Bordetella pertussis	Influenza B
Streptococcus pyogenes	Respiratory Syncytial Virus (*RSV)*
Hemophilus Influenza	Influenza A *H3N1*
Pseudomonas aeruginosa	*Corynebacterium diphtheria*

Analytical Sensitivity - Limit of Detection (LoD)

The Limit of Detection (LoD) is defined as the concentration (copies per µl of the eluate) of target molecule that can be detected at 95% or greater probability according to CLSI EP17-A2. The preliminary LoD of each target was determined by testing a 10-fold dilution series in triplicates per concentration, and then confirmed with 20 replicates of the concentration determined to be the LoD. The LoD assay of Hi-PCR® Adenovirus Probe PCR Kit was performed using 20 replicates each on Biorad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® AG Real Time PCR Systems using Quantitative Genomic DNA from Human adenovirus 1 strain Adenoid 71. The detectable limit of Hi-PCR® Adenovirus Probe PCR Kit was determined to be 8 copies / µL for Adenovirus genomic DNA

The analytical sensitivity in consideration with nucleic acid extraction was determined using a dilution series of the Quantitative Genomic DNA from Human Adenovirus 1 Strain: Adenoid 71, ATCC® Number: VR-1QD™ for nucleic acid amplification techniques from 1000 copies/µl to 1 copy/µl spiked into nasopharyngeal swabs collected in VTM. Experiments were carried out in triplicate, independent extractions (Sample volume: 200 µL, elution volume: 70 µL), followed by triplicate realtime PCR reactions for each extraction on HiMedia's InstaQ 96 AG series, Biorad's CFX Series and Thermo Fisher's QuantStudio™ 5 Real-Time PCR System. The analytical detection limit in consideration of the purification of Hi-PCR® Adenovirus Probe PCR Kit is **≈ 40 copies/µl.**

Evaluation

Each lot of Hi-PCR® Adenovirus Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® Adenovirus Probe PCR Kit is assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. Functionally tested in amplification.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions, and storage conditions have been used.
2.	Variability between replicates	Error in reaction set-up	Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a Real-Time PCR instrument.

		Pipetting error	C_t values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.
4.	No signal with positive controls	Incorrect programming of the temperature profile of the thermal cycler	Compare the temperature profile to the manual.

Safety Information

Hi-PCR® Adenovirus Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

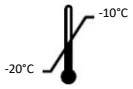
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures while disposing the infectious materials. Material that comes into contact with clinical samples must be decontaminated and disposed of in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Symbols:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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