RevoDx Adenovirus qPCR Kit

Instruction for Use

Qualitative detection of Adenovirus DNA

For in vitro diagnostic use

For professional use only

Product numbers:
IP202336-25 – 25 tests
IP202336-100 – 100 tests

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Doc. No / Rev. No : KK202336/ -
Issue Date / Rev. Date : 17.12.2021 / -

Product Components

	Component Name	25 Tests	100 Tests
1	AdV RM-1	350 µl	1400 µl
2	AdV RM-2	25 µl	100 µl
3	AdV Positive Control	100 µl	100 µl
4	AdV Negative Control	100 µl	100 µl

Transport, Storage and Stability

The kits may be shipped at +2°C to +8°C. All components of RevoDx Adenovirus qPCR Kit should be stored at -25°C to -15°C. Storage at higher temperatures should be avoided. If properly stored, all kit components are stable until the expiration date printed on the product label. Adenovirus RM 1 vial should not be freeze-thawed more than 3 times; as this may reduce the sensitivity. Otherwise, divide them into conveniently sized aliquots, and store at -25°C to -15°C.

Intended Use

RevoDx Adenovirus qPCR Kit is a real-time PCR test intended for the qualitative detection of Adenovirus DNA.
Negative results do not preclude Adenovirus infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.
RevoDx Adenovirus qPCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Product Use Restrictions

- For prescription use only
- RevoDx Adenovirus qPCR Kit is for in vitro diagnostic use only.
- Potential mutations in the target regions of the Adenovirus genome covered by the oligos in the kit may lead to false negative test results.
- PCR inhibitors in eluates may lead to false negative or invalid test results.
- Reliable results depend on proper specimen collection, transport, storage and handling methods.
- It is intended for professional use by properly trained personnel.
- Follow the instructions in product manual for optimum PCR results.
- Do not use a kit after its expiration date. Kit components from different lots should not be mixed.

Product Description

RevoDx Adenovirus qPCR Kit is a fluorogenic probe-based PCR assay in which, situated between two PCR primers, there is an internal oligonucleotide probe with a fluorescent label attached at the 5'-end and a quenching molecule that suppresses the fluorescent reporter at the 3'-end. During DNA replication in the PCR process, the internal oligonucleotide hybridizes to the template and is digested by the 5'-3' endonuclease activity of the Thermus aquaticus (Taq) DNA polymerase as the PCR primer is extended. The internal oligonucleotide is digested only if DNA replication occurs, separating the fluorescent and quencher molecules. PCR products are detected within minutes by monitoring the increase in fluorescence that occurs exponentially with successive PCR amplification cycles. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. RevoDx Adenovirus qPCR Kit utilizes an internal control, which controls for target isolation and amplification.

Instruments

The RevoDx Adenovirus qPCR Kit is to be used with BIO-RAD CFX96 and Tianlong Gentier 96 Real-Time PCR Detection Systems. But the RevoDx Adenovirus qPCR Kit may also be compatible with most real-time PCR detection systems with the channels FAM and HEX.

General Description

Adenoviruses (AdV) are a type of DNA virus that commonly cause mild infections in the upper or lower respiratory tract, gastrointestinal (GI) tract, or eyes. In rare cases, AdV infections can lead to more serious conditions such as hemorrhagic cystitis, hepatitis, hemorrhagic colitis, pancreatitis, nephritis, or encephalitis. Young children are more susceptible to AdV infections due to their underdeveloped immune systems. Epidemics of AdV infections can occur in closed or crowded environments, especially among military recruits. The disease can be more severe and likely to spread in individuals with weakened immune systems, such as organ transplant recipients, people with HIV/AIDS, or those with congenital immunodeficiency syndromes. Severe AdV pneumonia or disseminated disease, if left untreated, can have a fatality rate exceeding 50%. There are over fifty different serotypes of AdV, each with specific tissue preferences and associated clinical symptoms. The predominant serotypes vary by country or region and may change over time. It is also possible for new strains to emerge and spread between countries or continents, leading to the replacement of existing dominant serotypes.

Safety Information

- Clinical specimens should be treated as potentially infectious; they should be handled in Bio-safety Level 1 or Bio-safety Level 2 area, depending on the infective agents.
- All resulting waste should be considered potentially infectious. They should be handled and discarded according to local safety regulations.
- Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.
- Avoid producing spills or aerosol.
- Never pipette solutions by mouth
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands after handling samples and test reagents.
- All MSDS information is available upon request
- When working, always wear a protective lab coat, disposable gloves and protective goggles.
- Before and after procedure, disinfect all work surfaces thoroughly with a freshly prepared solution of 10% bleach or antiviral agents.
- Make sure everything is DNase/RNase-free when handling this system.
- Handle all materials according to Good Laboratory Practices in order to prevent cross-contamination.
- Use only calibrated pipettes, always change pipette tips between liquid transfers (aerosol-barrier pipette tips recommended)
- Keep the kit away from any source of contaminating nucleic acids, especially amplified nucleic acid.
- The operations should ideally be done in three separate areas. (i.e. for DNA/RNA purification, PCR setup, amplification) to prevent contamination.
- All equipment and consumables for a particular operation should be kept in the area where that operation is done and should not be moved between separated areas. Gloves should be removed and disposed of before leaving one area to proceed to the next. Lab coats should be specific to each area and never be worn outside the area.
- The work should flow in one direction, beginning in the extraction area followed by the chosen downstream application areas.

Performance Data

Analytical Sensitivity To determine the limit of detections (LoD), a dilution series of a secondary Adenovirus standard was prepared to give the final concentrations of 1000, 250, 45, 10 and 1.6 IU/ml. Viral DNA was purified using RevoDx Pathogen DNA/RNA Purification Kit. Each dilution was tested in 24 replicates. The Limit of Detection (LoD) value was found 73 IU/mL.

Diagnostic Specificity 105 Adenovirus DNA negative clinical specimens from individual donors were tested to determine the diagnostic specificity of RevoDx Adenovirus qPCR Kit. None of the tested samples gave positive test result for target. Diagnostic specificity of RevoDx Adenovirus qPCR Kit is ≥ 99 %.

Cross Reactivity The in silico analysis of the RevoDx Adenovirus qPCR Kit primers and probes against the sequences of 29 pathogens showed the kit would be specific to the target Adenovirus genes and not cross-react with these pathogens. The 15 pathogens listed below were wet tested with the RevoDx Adenovirus qPCR Kit for cross-reactivity. No false positive results were observed.
The results from the cross-reactivity, both in silico and wet testing, are summarized below.

In silico **Cross Reactivity Analysis**

Organism	Target oligos
Hepatitis C virus	No homology
Human Cytomegalovirus (HCMV)	No homology
Hepatitis B virus	No homology
SARS-CoV-2	No homology
Human coronavirus 229E	No homology
Human coronavirus OC43	No homology
Human coronavirus HKU1	No homology
Human coronavirus NL63	No homology
SARS-coronavirus	No homology
MERS-coronavirus	No homology
Human Metapneumovirus (hMPV)	No homology
Parainfluenza virus 1-4	No homology
Influenza A & B	No homology
Enterovirus (e.g. EV68)	No homology
Respiratory syncytial virus	No homology
Rhinovirus	No homology
Chlamydia pneumoniae	No homology
Haemophilus influenzae	No homology
Legionella pneumophila	No homology
Mycobacterium tuberculosis	No homology
Streptococcus pneumoniae	No homology
Streptococcus pyogenes	No homology
Bordetella pertussis	No homology
Mycoplasma pneumoniae	No homology
Pneumocystis jirovecii (PJP)	No homology
Candida albicans	No homology
Staphylococcus epidermis	No homology
Streptococcus salivarius	No homology

Wet Tested Cross Reactivity Analysis

Organism	Source	Result
Hepatitis C virus RNA for nucleic acid amplification techniques (6th WHO International Standard)	NIBSC (Cat. No: 18/184)	Not Detected
Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification Techniques (1st International Standard)	NIBSC (Cat. No: 09/162)	Not Detected
4th WHO International Standard for HBV DNA for NAT	NIBSC (Cat. No: 10/266)	Not Detected
First WHO International Standard for SARS-CoV-2 RNA	NIBSC (Cat. No: 20/146)	Not Detected
Human coronavirus (229E)	NIBSC (Cat. No: 09/132)	Not Detected
Rhinovirus	NIBSC (Cat. No: 08/324)	Not Detected
Influenza Virus (A/Christchurch/1/2003, H1N1)	NIBSC (Cat. No: 07/296)	Not Detected
Influenza Virus (A/Wyoming/3/2003, H3N2)	NIBSC (Cat. No: 07/298)	Not Detected
Influenza Virus (B/Jiangsu/10/2003)	NIBSC (Cat. No: 07/300)	Not Detected
Human Respiratory syncytial virus A2	NIBSC (Cat. No: 08/120)	Not Detected
Parainfluenza virus type 1	NIBSC (Cat. No: 08/176)	Not Detected
Parainfluenza virus type 2	NIBSC (Cat. No: 08/178)	Not Detected
Parainfluenza virus type 3	NIBSC (Cat. No: 08/118)	Not Detected
Parainfluenza virus type 4	NIBSC (Cat. No: 08/180)	Not Detected

Cross-Contamination The potential cross-contamination between samples was evaluated. Five different runs were performed by testing alternating high positive and negative samples 4 high positive Adenovirus sample and 4 Adenovirus negative samples were used in every run. No cross-contamination was observed, and none of the samples exhibited evidence of containing PCR inhibitors as indicated by the amplification of internal control.

Clinical Comparative Study Total 101 clinical samples were tested. According to the results, the data gathered by RevoDx Adenovirus qPCR Kit is compatible with the results of other CE-marked devices.

Additional Materials Required

- RevoDx Pathogen DNA/RNA Purification Kit (Cat. No: IP202302; idil biotech, Turkey) or RevoDx Magnetic Pathogen DNA/RNA Purification Kit (Cat. No: IP202303; idil biotech; Turkey)
- Real-Time PCR Detection System,
- Suitable protection (protective lab coat, disposable gloves, protective goggles, etc.)
- Micropipettes (0.5 µl – 1000 µl),
- DNase/RNase-free micropipette tips with filters,
- DNase/RNase-free 1.5 ml microcentrifuge tubes,
- Vortex mixer,
- Desktop microcentrifuge for PCR plates/strip tubes,
- Desktop microcentrifuge for 2.0 ml tubes,
- PCR Workstation,

Real-Time PCR reaction tubes or plates:

Protocol

Viral DNA Purification RevoDx Pathogen DNA/RNA Purification Kit or RevoDx Magnetic Pathogen DNA/RNA Purification Kit should be used for viral DNA extraction from clinical specimens. Using other purification kits may adversely affect the performance characteristics of the kit. Please follow the manufacturer's instructions as stated in the kit manual. The operations should ideally be done in three separate areas. (i.e. for DNA/RNA purification, PCR setup, amplification) to prevent contamination.

Internal Control An internal (Hs_RPP30) control targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. This also serves as the extraction control to ensure that samples resulting as negative contain nucleic acid for testing.

Positive Control Positive Control includes plasmid containing an insert. Positive Control is amplified in a separate Reaction Tube. To be able to evaluate the experiment, the Ct value of Positive Control should be equal to 26 ± 4, otherwise, it indicates a problem during amplification.

PCR Protocol

1. Thaw all components at room temperature except AdV RM 2. Put AdV RM 2 on ice. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.

2. The final volume of Master Mix is obtained by multiplying single reaction volumes of AdV RM 1 and RM 2 by the total sample size. When calculating the total sample size, the number of negative/positive controls and the clinical samples should be taken into consideration. For possible pipetting errors, it is recommended to add an extra sample to the total sample size.

3. To prepare master mix, add 14 µl of AdV RM 1 and 1 µl of AdV RM 2 for each sample to the master mix tube. Vortex the tube and spin down briefly in a microcentrifge. Add 15 µl of Master Mix into Real-Time PCR reaction tubes or capillaries for each sample. Add 5 µl DNA of each sample, negative control and positive control into the tubes. Spin down briefly.

4. Enter cycling conditions for Real-Time PCR Detection System: 95°C for 2 min, 1 cycle; 95°C for 10 sec, 60°C for 20 sec, 40 cycles (Table 3). Enter 20 µl as sample volume.

Table 3: Amplification program

Program Name	Cycles	Program
Hot Start	1	95ºC, 2 min
Amplification*	40	95ºC, 10 sec
		60ºC, 20 sec

*** Fluorogenic data should be collected at 60°C; FAM, and HEX channels should be chosen**

5. Fluorogenic data is collected at 60°C. FAM, and HEX channels should be selected.

6. Start run.

7. To program and analyze the results, refer to the User Manual of the instrument concerned.

Data Analysis

In order to evaluate the assay, the Ct value of Positive Control in the FAM channel must be equal to 26±4, and Negative Control in all channels must be negative. Otherwise, the experiment should be repeated.

The results can be interpreted as:

Signal in any FAM channel (Adenovirus DNA)	Signal in HEX channel (Internal Control)	Interpretation
+	+/-	Adenovirus DNA is positive
-	+	Target DNA is not detected
-	-	Invalid result. This sample should be re-tested

Ordering Information

Product Name	Package	Cat. No.
RevoDx Adenovirus qPCR Kit	25 tests	IP202336-25
RevoDx Adenovirus qPCR Kit	100 tests	IP202336-100