RevoDx Adenovirus / CMV / EBV qPCR Kit

Instruction for Use

Qualitative detection and identifcation of Adenovirus, Cytomegalovirus and Epstein-Barr virus DNA

For research use only

For professional use only

Product numbers:
IP202532-50 – 50 tests
IP202532-100 – 100 tests

Product Components

	Component Name	50 Tests	100 Tests
1	ACE MM-1	700 µl	1400 µl
2	ACE MM-2	700 µl	1400 µl
3	ACE Enzyme Mix	100 µl	200 µl
4	ACE Internal Control	150 µl	300 µl
5	ACE Positive Control	100 µl	100 µl
6	ACE 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 / CMV/ EBV 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. ACE MM vials 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/CMV/EBV qPCR Kit is an in vitro nucleic acid amplification test for qualitative detection and identification of Adenovirus, Cytomagalovirus and Epstein-Barr Virus DNA in human serum or plasma (EDTA) or cerebrospinal fluid (CSF) specimens.
Negative results do not preclude Adenovirus, CMV and Epstein-Barr Virus 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/CMV/EBV 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.

The following pathogens are detected by RevoDx Adenovirus/CMV/EBV qPCR Kit:

Viruses
• Adenovirus • Cytomegalovirus • Epstein-Barr Virus

Product Use Restrictions

- For prescription use only
- For research use only
- Potential mutations in the target regions of the Adenovirus, CMV and Epstein-Barr Virus genome covered by the oligos in the kit may lead to false negative test results.
- This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant or cerebrospinal fluid (CSF) specimens. Test with other sample types may result in inaccurate results.
- Plasma or serum samples from heparin treated blood are not suitable for use.
- 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/CMV/EBV 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/CMV/EBV qPCR Kit utilizes an internal control, which controls for target isolation and amplification.

Tube#	Target organism	Dye Channel
ACE MM 1	Adenovirus	FAM
	Epstein-Barr Virus	ROX
	Internal control	Cy 5
ACE MM 2	Cytomegalovirus	FAM
	Internal control	Cy 5

General Description

Epstein-Barr virus (EBV), a gamma herpesvirus, is a ubiquitous cause of infection in up to 95% of the general population (1). EBV is a DNA virus with a toroid-shaped protein core that is wrapped with DNA, a nucleocapsid with 162 capsomers, a protein tegument between the nucleocapsid and the envelope, and an outer envelope with external virus-encoded glycoprotein spikes. The EBV genome is a linear, double-stranded, ~172-kb DNA molecule that encodes > 85 genes (2). EBV infects B lymphocytes leading to their immortalisation, with persistence of the EBV genome as an episome (3). The disease can last several weeks and is characterized by lymphocytosis, sore throat, lymphadenopathy, and fatigue. Exposure to oral secretions during deep kissing has been identified as the major source for primary EBV infection in adolescents (4). Furthermore EBV has been associated with a remarkably diverse range of cancer types. Because EBV persists in the B cells of the asymptomatic host, it can easily be envisaged how it contributes to the development of B-cell lymphomas. However, EBV is also found in other cancers, including T-cell/natural killer cell lymphomas and several epithelial malignancies (5). It is reported that 80-86% of adults in Turkey are seropositive to EBV(6).

Adenoviruses are a group of viruses that primarily lead to infections of the upper and lower respiratory tract. They are also capable of causing a range of other illnesses, such as gastrointestinal, neurological, and ocular infections. Recently, outbreaks of hepatitis—including severe liver failure in children reported in Europe and the United States—have been linked to adenovirus infection. While adenoviruses usually result in mild illness, certain cases can progress to severe disease and even death. Current investigations are ongoing, as adenovirus alone may not fully account for the most severe cases observed.

Cytomegalovirus (CMV) is a common infection caused by a herpesvirus. If a person becomes infected shortly before or during pregnancy, the virus can be transmitted to the baby, leading to congenital CMV. This condition may cause hearing loss, developmental delays, and other health problems in the child.CMV can also lead to serious complications in individuals with weakened immune systems, such as organ transplant recipients or people undergoing immunosuppressive therapy.

References

1. Nowalk A, Green M. Epstein-Barr Virus. Microbiol Spectr. 2016 Jun;4(3). doi: 10.1128/microbiolspec.DMIH2-0011-2015. PMID: 27337443.
2. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Biological Agents. Lyon (FR): International Agency for Research on Cancer; 2012. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 100B.) EPSTEIN-BARR VIRUS.
3. Kerr JR. Epstein-Barr virus (EBV) reactivation and therapeutic inhibitors. J Clin Pathol. 2019 Oct;72(10):651-658. doi: 10.1136/jclinpath-2019-205822. Epub 2019 Jul 17. PMID: 31315893.

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

Limit of Detection (LoD) - Analytical Sensitivity Study:

To determine the limit of detections (LoD), a dilution series of WHO International Adenovirus/CMV/EBV standard was prepared to give the final concentrations of 160, 80, 40, 20, 10 and 5 IU/ml. Each dilution was tested in 24 replicates. Limit of Detection (LoD) values were calculated by probit analysis The Limit of Detection (LoD) value was 85 IU/mL

Inclusivity:

An *in silico* inclusivity analysis of the RevoDx Adenovirus/CMV/EBV qPCR Kit primers and probes was performed for the sequences of each pathogen available from NCBI databases. The alignments demonstrated that the regions recognized by the designed primers and probes have 100% homology with all available pathogen sequences from the National Center for Biotechnology Information (NCBI) databases/databanks.

Cross Reactivity:

Cross-reactivity of the RevoDx Adenovirus/CMV/EBV qPCR Kit was evaluated using both in silico analysis and by wet testing. The in silico analysis of the RevoDx Adenovirus/CMV/EBV qPCR Kit primers and probes against the sequences of 3 pathogens showed the kit would be specific to the specific targets and not cross-react with these pathogens. The 22 pathogens listed below were wet tested with the RevoDx Adenovirus/CMV/EBV 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	Result
Bacillus subtilis	No homology
Mycobacterium tuberculosis	No homology
Streptococcus salivarius	No homology
Pneumocystis jirovecii (PJP)	No homology
Entamoeba dispar	No homology
Proteus spp.	No homology
Saccharomyces cerevisiae	No homology
Schizosaccharomyces pombe	No homology
Aspergillus niger	No homology
Salmonella spp.	No homology
Serratia marcescens	No homology
JC virüs	No homology
BK virüs	No homology
Parvovirus B19	No homology
Human Norovirus	No homology
VZV	No homology
HIV-1	No homology
HIV-2	No homology
HCV	No homology
HBV	No homology
Ebola virüs	No homology
Human Parechovirus	No homology

Wet Tested Cross Reactivity Analysis

Organism	Source	Concentration	Result
Pneumocystis jirovecii (PJP)	Clinical specimen	no unitage assigned	Not Detected
Entamoeba dispar	Clinical specimen	no unitage assigned	Not Detected
Mycobacterium tuberculosis	Clinical specimen	no unitage assigned	Not Detected
Aspergillus niger	Clinical specimen	no unitage assigned	Not Detected
Measles Virus	Clinical specimen	no unitage assigned	Not Detected
Candida albicans	Clinical specimen	no unitage assigned	Not Detected
Cryptococcus neoformans	Clinical specimen	no unitage assigned	Not Detected
Escherichia coli	Clinical specimen	no unitage assigned	Not Detected
Neisseria meningitidis	Clinical specimen	no unitage assigned	Not Detected
Legionella feeleii	Clinical specimen	no unitage assigned	Not Detected
Klebsiella pneumoniae	Clinical specimen	no unitage assigned	Not Detected
Chlamydia trachomatis	Clinical specimen	no unitage assigned	Not Detected
Mycoplasma hominis	Clinical specimen	no unitage assigned	Not Detected
Neisseria gonorrhoeae	Clinical specimen	no unitage assigned	Not Detected
Human Immunodeficiency Virus 1 (HIV-1)	NIBSC (Cat. No: 16/194)	1.25×10^5 IU/ml	Not Detected
Human Immunodeficiency Virus 2 (HIV-2)	NIBSC (Cat. No: 16/296)	2.8×10^5 IU/ml	Not Detected
4th WHO International Standard for HBV DNA for NAT	NIBSC (Cat. No: 10/266)	9.55×10^5 IU/ml	Not Detected
Hepatitis C virus RNA (6th WHO International Standard)	NIBSC (Cat. No: 18/184)	2.57×10^5 IU/ml	Not Detected
VZV (1st WHO International Standard)	NIBSC (Cat. No: 19/164)	1×10^7 IU/ml	Not Detected
EBOV RNA NP-VP35-GP (WHO Reference Reagent)	NIBSC (Cat. No: 15/222)	no unitage assigned	Not Detected
Parvovirus B19 (1st International Standard)	NIBSC (Cat. No: 09/110)	9.55×10^5 IU/ml	Not Detected
HSV-1	NIBSC (Cat. No: 16/368)	no unitage assigned	Not Detected
HSV-2	NIBSC (Cat. No: 17/122)	no unitage assigned	Not Detected
JC Virus (JCV) DNA (1st International Standard)	NIBSC (Cat. No: 14/114)	1.55×10^7 IU/ml	Not Detected
BK Virus (BKV)(1st International Standard)	NIBSC (Cat. No: 14/122)	2.04×10^7 IU/ml	Not Detected
HHV-6 Virus 1st WHO International Standard	NIBSC (Cat. No: 15/266)	5.63×10^7 IU/ml	Not Detected
Human Parechovirus	NIBSC (Cat. No: 08/322)	no unitage assigned	Not Detected
Human Norovirus	NIBSC (Cat. No: 08/318)	no unitage assigned	Not Detected
First WHO International Standard for Mycobacterium tuberculosis	NIBSC (Cat. No: 20/152)	2×10^6 IU/ml	Not Detected

Clinical Evaluation:

108 Adenovirus/CMV/EBV DNA negative clinical specimens from individual donors were tested to determine the diagnostic specificity of RevoDx Adenovirus/CMV/EBV qPCR Kit. 52 Adenovirus/CMV/EBV DNA negative clinical serum specimens and 56 Adenovirus/CMV/EBV DNA negative clinical EDTA plasma specimens were used. None of the tested samples gave positive test result for target. Diagnostic specificity of RevoDx Adenovirus/CMV/EBV qPCR Kit is ≥ 99 %. According to the test results, 100% agreement was observed with expected results.

Additional Materials Required

- RevoDx Viral Nucleic Acid Purification Kit (Cat. No: IP201906; idil biotech,Turkey)
- RevoDx Magnetic Viral Nucleic Acid Purification Kit (Cat. No: IP201920; 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,
- PCR Workstation,
- Real-Time PCR reaction tubes or plates,

Sample Preparation

This kit has been validated for use with fresh or frozen human serum or plasma collected in EDTA anticoagulant or cerebrospinal fluid (CSF) specimens. Plasma or serum samples from heparin treated blood are not suitable for use. Clinical specimens should be treated as potentially infectious; and blood-borne pathogen precautions are recommended during sample collection and handling. Clinicians (including healthcare assistants, nurses, doctors and professionals allied to medicine) have the responsibility of using the correct procedure during the collection and safe transportation of samples to the laboratory. The validity of test results largely depends on good practice in the 'pre-test' stage and it is essential that documentation is accurate and comprehensive.

After collecting, do not store the whole blood at room temperature for longer than 4 hours. Centrifuge blood and transfer serum or plasma to a screw cap cryovial tube. Transportation of whole blood, serum or plasma must conform to country or local regulations. Serum or plasma samples may be stored at 2-8°C for up to 24 hours or frozen at -70°C or colder for long-term storage. Repeated freeze/thaw cycles must be avoided because this will result in a decrease of the virus titer.

Samples must be mixed by inverting the tubes or pipetting several times before transferring to the sample tube. If using frozen samples, equilibrate samples to room temperature before starting the procedure. Remove precipitates, if any, by centrifugation for 3 min at 5,000 x g.

Protocol

Viral DNA Purification RevoDx Viral Nucleic Acid Purification Kit should be used for viral DNA extraction from human serum or plasma collected in EDTA anticoagulant or cerebrospinal fluid (CSF) 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 The presence of the internal control (IC) during the purification procedure is necessary. Internal Control includes plasmid DNA containing an insert. The internal control is utilized to monitor the efficiency of DNA extraction step as well as to check any PCR inhibition. For each sample, add 2.5 µl IC into Lysis Solution of RevoDx Viral Nucleic Acid Purification Kit. **Do not add IC directly into clinical sample.** Depending on final elution volume, the volume of IC to be added is calculated (0.05 µl IC/1 µl Elution Buffer). Bad signal or no signal might be observed in the internal control channel where high positive samples are amplified, because there is a competition between internal control template and target template while using PCR components. The Ct value of the internal control of a negative sample should be equal to 28 ± 4, otherwise, it indicates a problem during purification.

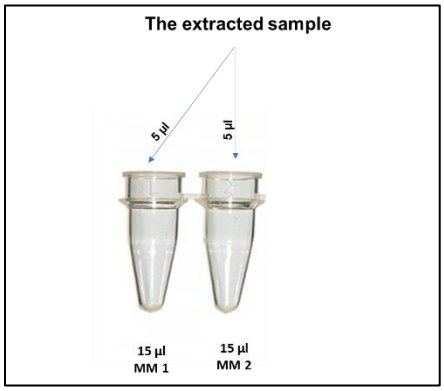
Positive Control: To be able to evaluate the experiment, the Ct values of Positive Control should be equal to 26 ± 4, otherwise, it indicates a problem.

PCR Protocol

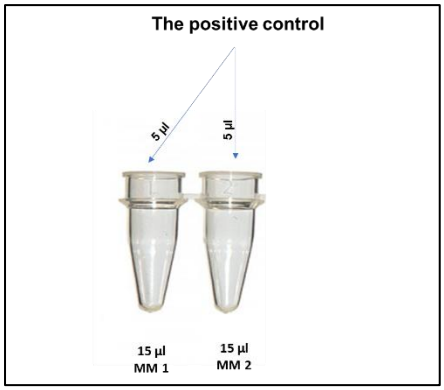
1. Thaw all components at room temperature except ACE Enzyme Mix. Put ACE Enzyme Mix on ice. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.

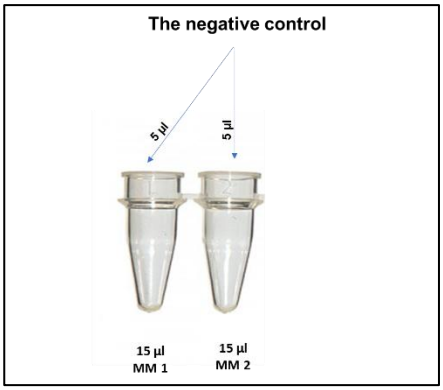
2. The final volumes of Master Mixes are obtained by multiplying single reaction volumes of any ACE MM and ACE Enzyme Mix by the total sample size. When calculating the total sample size, the number of negative controls, positive controls and the clinical samples should be taken into consideration. For possible pipetting errors, it is recommended to add an extra sample to the total sample size.

3. To prepare each master mix, add 14 µl of ACE MM and 1 µl of ACE Enzyme Mix for each sample to the master mix tube. After preparing Master Mixes, vortex the tubes gently and spin down briefly. Add 15 µl of each Master Mix to PCR reaction tubes/plate. For each clinical specimen, 2 wells should be used. After the additions of Master Mixes into the wells, add 5 µl of the extracted sample into each well as shown in figure below. Close the cap of 8-Well Strips or seal the plate. Spin down briefly.



4. Repeat Step 3 for each extracted sample, negative control and positive control.





5. 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 1). Enter 20 µl as sample volume.

Table 1: 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, ROX and Cy 5 channels should be chosen**

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

7. Start run.

8. 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 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 for each Master Mix as:

Signal in any FAM / ROX channel	Signal in Cy 5 channel (RNase P gene)	Interpretation
+	+/-	Positive for specific pathogen
-	+	Pathogen is not detected
-	-	Invalid result. This sample should be re-tested for this Master Mix

For each Master Mix, the dye channels of the target organism/target gene are given in the following table:

Tube#	Target organism	Dye Channel
ACE MM 1	Adenovirus	FAM
	Epstein-Barr Virus	ROX
	Internal control	Cy 5
ACE MM 2	Cytomegalovirus	FAM
	Internal control	Cy 5

Ordering Information

Product Name	Package	Cat. No.
RevoDx Adenovirus/CMV/EBV qPCR Kit	50 tests	IP202532-50
RevoDx Adenovirus/CMV/EBV qPCR Kit	100 tests	IP202532-100