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CAUTION! Professional use only.
Follow instruction manual before use.
Reading and carefully following these warnings will minimize usage errors, other possible risks and hazards.
Wear safety glasses, clothes, and gloves.

ADENOVIRUS RT-qPCR DETECTION KIT

User Guide

Catalog Number	Pack Size
ZT-ADV-01-100	100 Tests
ZT-ADV-01-500	500 Tests
ZT-ADV-01-1000	1000 Tests

Content and Storage Condition

Content	Description	Quantity (20µL/Reaction)			Storage Condition	Routine Storage Condition
		100 Tests	500 Tests	1000 Tests		
SOFTEC RT-qPCR Mix	Ready-to-use reaction mixture for RT-qPCR	1,9 mL	5 x 1,9 mL	10 x 1,9 mL	-20°C	-20°C
SOFTEC PC	Positive Control	0.05 mL	0.05 mL	0.1 mL	-20°C	-20°C
SOFTEC NC	Negative Control	0.05 mL	0.05 mL	0.1 mL	-20°C	-20°C

Features

- The kit contents should be stored at -20°C. It can be stored until the expiration date.
- In order for SOFTEC RT-qPCR to work with full efficiency, the maximum freeze-thaw number is 5.
- To avoid repeated freezing and minimize the risk of contamination of stock solutions, it is strongly recommended to divide high-volume stocks into smaller volumes and store them in accordance with the storage condition (-20°C).
- SOFTEC ADENOVIRUS RT-qPCR Kit is compatible with both types of qPCR devices that require and do not require reference dye for fluorescent signal normalization.
- The effectiveness of the kit was determined by DNA isolated from human nasopharyngeal swab, oropharyngeal swab, and whole blood samples.

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Product Description

Adenovirus is a group of viruses that cause respiratory, digestive and eye infections in many living species. Human adenoviruses (HAdV) are known up to 51 serotypes, and some of these serotypes can cause mild colds, sore throats, eye infections and diarrhea, while others can lead to serious respiratory infections, pneumonia, meningitis, and deadly diseases.

SOFTEC Adenovirus RT-qPCR Detection Kit is used for qualitative detection of Adenovirus with DNA isolated from human nasopharyngeal swab, oropharyngeal swab, and whole blood samples. The sensitivity and accuracy optimizations of the kit were performed by real-time one-step PCR experiments using hydrolysis probes of adenovirus DNA targets.

The working principle of the SOFTEC Adenovirus RT-qPCR Detection Kit is based on the detection of fluorescent signals in the presence of target nucleotide sequences of DNA. PCR for this process takes place in 3 steps. First step: meltdown. In this step, the DNA becomes highly denatured, and its strands separate. Step two: bonding. In this step, the primers are attached to their target sequences on the DNA. Step three: elongation. In this step, the primers that hold on to the target sequences are extended. These described steps are for a PCR cycle. The cycle repeats until an efficient and sufficient concentration is achieved. Adenovirus-specific primers are used for the amplification of target sequences and target-specific probes are used for the detection of amplified DNA. The probes are oligonucleotides with a fluorescent probe attached to the 5' end and an extinguisher attached to the 3' end. The adenovirus-specific probe is labeled with fluorophore FAM. The probe specific to internal control is labeled with fluorophore HEX. During elongation, DNA polymerase separates the reporter and the extinguisher. With this separation, the fluorescence signal is emitted and measured in real time.

SOFTEC Adenovirus RT-qPCR Detection Kit reaches the user in a single tube that contains all the necessary components to perform RT-qPCR.

Adenovirus RT-qPCR Detection Kit Protocol

Clean the workbench to be worked with 0.5-1% (w/v) sodium hypochlorite first, then with 70% Ethyl alcohol. Thaw the reagents at 4°C on a cold shelf or on ice. If your time for work is limited, thaw them at room temperature. Mix each reagent several times by gently pipetting up and down, then centrifuge briefly. To avoid many freeze-thaw processes, please divide the mixes into sterile dnase/rnase free tubes at the first use. Prevent the fluorescent marked probes in the SOFTEC Adv Mix from being exposed to light and perform the operation in a dark environment every time you use it. Determine the total number of reactions per assay run. Each assay run should include the following:

One Positive Control Sample that uses the PC (Positive Control) provided in the kit, as template.
One Negative Control Sample that uses the NC (Negative Control) provided in the kit, as template.
Total the number of Collected Samples

Component	Volume Per 20 µl Reaction		
	Positive Control	Negative Control	Test Sample
SOFTEC RT-qPCR Mix	19 µL	19 µL	19 µL
Test Sample	-	-	6 µL
PC	6 µL	-	-
NC	-	6 µL	-

Run your RT-qPCR assay as shown in table below. NOTE: When programming your RT-qPCR run, choose the detection channel(s) as FAM and HEX.

Target	Fluorophore/Channel
ADENOVIRUS Target Gene	FAM
Internal Control Gene	HEX

Data Analyses

Before starting the analysis, the threshold value for Bio-Rad brand devices, FAM and HEX channel should be set to 200 RFU.

	FAM	HEX	Interpretation
PC	Ct < 33	Ct < 33	Positive
NC	-	Ct < 33	Negative
Test Samples	Ct < 33	Ct < 33	Positive
	-	-	Invalid Test
	-	Ct < 33	Negative

Safety and Hazards

General Safety

Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

General Requirements for Good Practices on PCR and RT-QPCR

Laboratory Setup

To prevent contamination of the reaction mixture by previously amplified target sequences, it shall be ensured that separate work areas with their own apparatus are available. If possible, maintain separate work areas, dedicated equipment, and supplies for: Sample preparation, PCR setup, PCR amplification, Analysis of PCR products.

Personnel

SOFTEC Adenovirus RT-qPCR Detection Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the molecular techniques and in vitro diagnostic procedures. Different sets of laboratory coats should be worn pre- and post-PCR. Disposable gloves should be worn at sample preparation and when setting up RT-qPCR.

Protection of Product Performance and Analysis Efficiency

The components in the kit should not be mixed with components with different lot numbers or chemicals of the same name but from different manufacturers. Master stock reagents should be kept on the cold block during the
PCR setup: if possible, the PCR setup should be performed on the cold block. Kit components should be mixed by gently shaking before use.

Preventing Contamination

The kit should be stored away from nucleic acid sources and qPCR amplicons. The micropipettes used for pipetting qPCR mixes and template nucleic acids should be separate. Filtered and nuclease-free pipette tips should be used.

Plate Layout Suggestions

In multi-targeted PCR runs, separate different targets by a row or by a column if enough space is available. If possible, put at least one well between unknown samples and controls.

Warning

Do not preserve the product when the package is damaged.

Troubleshooting

Observation	Possible Cause	Recommended Action
Failure to detect FAM/HEX signals in Positive Control wells or HEX signals in Negative Control wells	PCR amplification failure	Make sure that the mix and PC/NC samples are pipetted correctly. Check that the thermal cycler settings and amplification program are correct. If there are no errors in them, renew the reagents and repeat the reaction. Contact with the manufacturer.
In the Negative Control wells, target-specific signals (FAM) are detected.	Contamination of the PCR	The cause of contamination may be due to hand errors made in sample processing, reagent contamination or environmental factors. Decontaminate the benchtop surfaces and other equipment where the PCR process is performed with 70% Ethyl alcohol and repeat the PCR process. Pipette Positive Control reactions last to avoid cross-contamination. Be sure to pay attention to the points in the section "General Requirements and Warnings on PCR and RT-qPCR Good Practices".
No signal is detected in any channel in the test sample wells	Inhibition Problem	Dilute the test sample in a ratio of 1:10 and repeat the PCR procedure. If the diluted sample does not show a positive result in the HEX channel, request a new sample from the patient. If the problem persists, contact with the manufacturer.

Assay Limitations

- The kit is intended for use in a laboratory environment by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- A false negative result may occur if a specimen is improperly collected, transported, or handled.
- Performance of the kit has only been established in nasopharyngeal swab, oropharyngeal swab, nasal swab, and oral/saliva swab specimens suspected of respiratory tract viruses' samples.

Limitations of the Test

- SOFTEC ADENOVIRUS RT-qPCR Detection Kit should not be used by untrained personnel.
- If a sample is not collected, transported or processed properly, a false negative result may occur. In this case, the kit manufacturer company does not accept responsibility.
- The performance of SOFTEC ADENOVIRUS RT-qPCR Detection Kit is optimized only with DNA isolated from human nasopharyngeal swab, oropharyngeal swab and whole blood samples.
- Viral DNA detection may be affected by patient factors and/or the stage of infection. The kit manufacturer company cannot be held responsible for the different results that may result from this situation.