softec

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.

EBOLA ZAIRE RT-qPCR KIT

User Guide

Catalog Number	Pack Size
ZT-EBOV-01-200	200 x 20 µL reactions
ZT- EBOV -01-1000	1000 x 20 µL reactions
ZT- EBOV -01-2000	2000 x 20 µL reactions

Content and Storage Condition

Content	Quantity	Shipping Condition	Routine Storage Condition
SOFTEC Enzyme Mix	1,76 ml x 10 pcs	Temporary storage for up to 1 month at room temperature	-20°C
SOFTEC Oligo Mix	1,24 ml x 10 pcs	-20°C	-20°C
Positive Control	0.05 mL	-20°C	-20°C
Negative Control	0.05 mL	-20°C	-20°C

Features

- Kit content must be stored at -20°C until expiry date.
- Freeze thaw cycles for SOFTEC Enzyme Mix and SOFTEC Oligo Mix are up to 30 times.
- To avoid repeated freezing and thawing as well as to minimize the contamination risk of stock solutions of reagents, it is highly recommended to divide large-volume stocks into several smaller aliquots and store them at -20°C.
- Kit is compatible with both qPCR cyclers that do not require and require an internal reference dye for normalization of fluorescent signal.

Product Description

Ebola virus was discovered in 1976 in different parts of Central Africa. Ebola virus is in the family of Filoviridae including five known distinct species: Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Reston ebolavirus (RESTV), Bundibugyo ebolavirus (BDBV), Tai Forest ebolavirus (TAFV). The Filoviruses' genomic structure is homologous, and it is approximately 19 kb long single-stranded, negative-sense RNA containing seven genes: RNA polymerase (L), Nucleoprotein (NP), viral protein (VP) 35, VP40, Glycoprotein (GP), VP30, VP24.

Ebola virus is very efficient to invade immune system by attacking and damaging macrophages and dendritic immune cells. By this way Ebola invade many other organs. Ebola virus causes a fatal viral

Mix the reaction mix thoroughly, then centrifuge briefly.
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.

Steps		Temperature	Time
Reverse transcription		50°C	15 min
Enzyme activation		95°C	10 min
40 Cycles	Denaturaton	95°C	15 sec
	Annealing/Extension	60°C	60 sec

Collect and analyze the data according to the instrument- specific instructions. Verify the amplification curve.

Target	Fluorophore/Channel
Ebola Zaire target gene	FAM
Internal 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 < 35	Ct < 35	Positive
NC	-	-	Negative
Test Samples	Ct < 35	Ct < 35	Positive
	-	-	Invalid Test
	Ct < 35	-	Positive (Severe Ebola Zaire infection)
	-	Ct < 35	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 Ebola Zaire RT-qPCR 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 set up; 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.

Assay Limitations

- SOFTEC Ebola Zaire RT-qPCR 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.
- The clinical specimens shall be collected by a healthcare provider when working with Ebola Zaire in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for Ebola Zaire.
- A false negative result may occur if a specimen is improperly collected, transported, or handled.
- Performance of SOFTEC Ebola Zaire RT-qPCR Kit has only been established in nasopharyngeal swab, oropharyngeal swab, nasal swab, and oral/saliva swab specimens suspected of respiratory tract viruses' samples.
- Detection of viral RNA may be affected by patient factors and/or stage of infection.

Troubleshooting

Observation	Possible Cause	Recommended Action
In the Positive Control wells, no target-specific and no Internal Control signals are detected.	PCR amplification failure.	Check that the thermal cycler settings and amplification program are correct. If there is no error in these, there may be a reagent problem; contact the manufacturer, renew the reagents, and repeat the reaction.
In the Negative Control wells, target-specific and/or IC signals are detected.	Contamination of the PCR.	Contamination may be due to errors in sample handling, reagent contamination, or environmental contamination. Decontaminate benchtop surfaces and other equipment where PCR is performed with 10% bleach solution. Use fresh reagents and repeat the PCR. Set up the Positive Control reactions last to avoid cross-contamination. Pay attention to the issues in the "General Requirements and Warnings for Good Practices on PCR and RT-qPCR" section.
In unknown wells (sample wells), no internal control signal is detected, but target-specific signal is detected.	A high copy number of target nucleic acid exists in samples, resulting in preferential amplification of the target-specific NA.	No action is required. The result is considered positive.
In sample wells, no Internal Control and no target-specific signal is detected	Inhibition Problem	Dilute the nucleic acid isolate 1/10 and repeat the PCR. If the diluted sample does not give a positive result in the Internal Control channel, request for a new sample and repeat the nucleic acid extraction. If the problem persists, contact Technical Support.

hemorrhagic fever. The symptoms for Ebola virus disease (EVD) comprise fever, muscle pain, headache, vomiting, diarrhea, rash, internal and external bleeding.

SOFTEC Ebola Zaire RT-qPCR Kit is the qualitative detection and identification of the selected target gene within the genomes of Zaire Ebola Virus isolated from blood, plasma or serum. Kit principle is based upon the transcription of RNA to complementary DNA (cDNA) using reverse-transcriptase (RT). This cDNA is used as template for real time RT-qPCR. The cDNA target sequences are amplified utilizing DNA polymerase by PCR process. PCR has three steps: the first step is melting, DNA is denatured at high temperature yielding to single stranded; the second step is annealing, target specific primers anneal to the target sequence in cDNA; the last and third step is elongation that primers attached to target sequences are extended by DNA polymerase. This is for one PCR cycle and until obtaining efficient DNA concentration the cycle repeats. Ebola Zaire specific area-based primers for amplification of target sequences and target specific probes are used for the detection of amplified DNA. Probes are oligonucleotides that have fluorescent probe attach to the 5'end, and quencher to 3'end. Ebola Zaire specific probes are labelled with fluorophore FAM. Internal control specific probe is labelled with the fluorophore VIC/HEX. Throughout the elongation, DNA polymerase cleaves the reporter and quencher by this separation the fluorescence is emitted and measured.

SOFTEC Ebola Zaire RT-qPCR Kit comes in 4 different tubes and contains all the components necessary to perform RT-qPCR in a single tube on qPCR cyclers

SOFTEC EBOLA ZAIRE RT-qPCR KIT

Thaw the reagents at room temperature. Mix each reagent by gentle vortex or pipetting up and down, then centrifuge briefly. Place in a cold rack at 4°C or ice. NOTE: Minimize the exposure of the fluorescently labelled probe(s) to light.

Determine the total number of reactions per assay run. Each assay run should include the following:

i. One Positive Control using the EBOLA ZAIRE Positive Control provided in the kit, as template.
ii. One Negative Control using Nuclease-free Water provided in the kit, as template
iii. Total the number of Collected Samples.

Component	Volume Per 20 µl Reaction		
	Positive Control	Negative Control	Test Sample
5X SOFTEC Enzyme Mix	8,8 µL	8,8 µL	8,8 µL
5X SOFTEC Oligo Mix	6,2 µL	6,2 µL	6,2 µL
Test Sample	5 µL	-	-
Positive Control	-	5 µL	-
Negative Control	-	-	5 µL