Primer Design Ltd

R00965

Ebola Virus:

Sudan and Zaire Strains

Kit version: 1

Target region for both strains:

Nucleoprotein (NP) gene

genesig® Advanced Kit

150 tests

GENESIG

Kits by Primerdesign

Specificity of primers and probes last reviewed on:
07 December 2022

For general laboratory and research use only

Introduction to Ebola virus

Ebola viruses are the causative agents for a severe, lethal viral disease called Ebola haemorrhagic fever in humans and primates. They are long, filamentous, enveloped and non-segmented viruses with a negative stranded RNA genome, belonging to the family Filoviridae. The genus ebolavirus is comprised of six distinct species: Bundibugyo, Bombali, Tai Forest (formerly Côte d'Ivoire), Reston, Sudan and Zaire, although Reston and Bombali ebolavirus are not thought to cause illness in people. They are tubular virions, generally 80 nm in diameter and 800 nm in length. The genome is approximately 19 kb long and consists of 7 linearly arranged genes in order 3'- NP-VP35-VP40-GP-VP30-VP24-L. The first recorded outbreak of Zaire ebolavirus occurred in Yambuku, Zaire in 1976. In the same year, the Sudan ebolavirus was identified, though initially believed to be identical to the Zaire species.

The Ebola virus is primarily transmitted to humans through close contact with the blood, secretions, organs or other bodily fluids of infected animals. It then spreads into the community through human-to-human transmissions, resulting from direct contact with the blood or secretions of an infected person. Initially it is commonly spread among family and friends of infected individuals. Health-care workers have frequently been infected while treating Ebola patients and outbreaks in hospital environments are common. Transmission via infected semen can occur up to seven weeks after clinical recovery. Decomposed bodies of infected individuals can remain infectious for 3 to 4 days after death.

The incubation period of Ebola haemorrhagic fever is usually 5 to 20 days. Clinical signs of the infection include fever, headache, sore throat, joint and muscle aches, diarrhoea and weakness. It is a febrile haemorrhagic illness where the case-fatality rate varies between 25% and 90%. In severe cases of haemorrhagic fever, there can be damage to blood vessels and extensive internal and external bleeding or haemorrhage. Zaire ebolavirus has the highest case-fatality rate among all Ebola viruses. It can be up to 90% in some epidemics, with an average case fatality rate of approximately 83% over 27 years. There have been more outbreaks of Zaire ebolavirus than of any other species. In recent years, therapeutic treatments and vaccines have been developed which may increase survival rate in patients. Ebola outbreaks have continued to emerge periodically; in 2022, 6 cases of Zaire ebolavirus infection were announced, with 100% mortality rate. 164 cases of Sudan ebolavirus were also reported in 2022, with a mortality rate of 34%.

Specificity

The genesig®Advanced Kit for Ebola virus (Zaire and Sudan) is designed for the in vitro quantification of these two Ebola virus genomes, which have been responsible for the majority of outbreaks. The kit is designed to have a broad detection profile. Specifically, the primers will detect over 95% of the sequences on the NCBI Genbank database, identified as Ebola virus of Zaire or Sudan origin and collected within the three years to the time of the last review. Due to the sequence evolution observed in the genomes of RNA viruses, a period of three years is considered an adequate time period for analysis.

The EBOV_S primer and probe set is designed to detect only Sudan ebolavirus sequences and to exclude detection of other Ebola virus species.

The EBOV_Z_v2.0 primer and probe set is designed to detect only Zaire ebolavirus sequences and to exclude detection of other Ebola virus species.

The dynamics of genetic variation mean that new sequence information may become available after the most recent review. Primer Design Ltd periodically reviews the detection profiles of our kits and when required releases new versions.

If you require further information or have a specific question about the detection profile of this kit then please send an e-mail to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

- **1x EBOV_S primer/probe mix (150 reactions, BROWN)**
  FAM labelled

- **1x EBOV_Z-v2.0 primer/probe mix (150 reactions, BROWN)**
  FAM labelled

- **1x EBOV_S positive control template (RED)**

- **1x EBOV_Z-v2.0 positive control template (RED)**

- **2x Internal extraction control primer/probe mix (150 reactions, BROWN)**
  VIC labelled as standard

- **1x Internal extraction control RNA template (150 reactions, BLUE)**

- **1x Endogenous control primer/probe mix (150 reactions, BROWN)**
  FAM labelled

- **1x RNase/DNase free water (WHITE)**
  for resuspension of primer/probe mixes

- **3x Template preparation buffer (YELLOW)**
  for resuspension of internal control template, positive control template and standard curve preparation

Reagents and equipment to be supplied by the user

Real-time PCR Instrument Extraction kit
This kit is recommended for use with genesig®Easy DNA/RNA Extraction kit or exis®Mag. However, it is designed to work well with all processes that yield high quality RNA and DNA with minimal PCR inhibitors.

oasig® lyophilised OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix
This kit is intended for use with oasig® lyophilised OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix

Pipettors and filter tips

Vortex and centrifuge

1.5 ml tubes

qPCR plates or tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20°C on arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.
If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.
Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA integrity (An internal PCR control is supplied to test for non-specific PCR inhibitors). Always run at least one negative control with the samples. To prepare a negative control, replace the template RNA sample with RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template. If running a positive control standard curve for a quantitative result, and an efficiency of between 90% to 110% is not achieved, then the run should be repeated with a freshly prepared standard curve.

Notices and disclaimers

This product is developed, designed, and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks

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Principles of the test

Real-time PCR

EBOV_S and EBOV_Z-v2.0 primer/probe mixes are provided, and these can be detected through the FAM channel.

The primer/probe mixes provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

For copy number determination and as a positive control for the PCR set up, the kit contains positive control templates. This can be used to generate standard curves of target copy number/Cq value. Alternatively, the positive controls can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target genes worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive controls do not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control wells.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC.

Internal RNA extraction control

When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration.

A separate qPCR primer/probe mix is supplied with this kit to detect the exogenous RNA using qPCR. The PCR primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control cDNA does not interfere with detection of the target cDNA even when present at low copy number. The Internal control is detected through the VIC channel and gives a Cq value of 28+/-3 depending on the level of sample dilution.

Endogenous control

To confirm extraction of a valid biological template, a primer/probe mix is included to detect an endogenous gene. Detection of the endogenous control is through the FAM channel, and it is NOT therefore possible to perform a multiplex with the target primers. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Resuspension protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure the lyophilised primer/probe mix is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mixes in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in water	Volume
Pre-PCR pack	
EBOV_S primer/probe mix (**BROWN**)	165 µl
EBOV_Z-v2.0 primer/probe mix (**BROWN**)	165 µl
Internal extraction control primer/probe mix (**BROWN**)	165 µl
Endogenous control primer/probe mix (**BROWN**)	165 µl

3. **Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Pre-PCR heat-sealed foil	
Internal extraction control RNA template (**BLUE**)	600 µl
Post-PCR heat-sealed foil	
EBOV_S Positive Control Template (**RED**) *	500 µl
EBOV_Z-v2.0 Positive Control Template (**RED**) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

RNA extraction

The internal extraction control RNA can be added either to the RNA lysis/extraction buffer or to the RNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control RNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. **Add 4µl of the Internal extraction control RNA (BLUE) to each sample in RNA lysis/extraction buffer per sample.**

2. **Complete RNA extraction according to the manufacturer's protocols.**

OneStep RT-qPCR detection protocol

For optimum performance and sensitivity.
All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared proceed immediately to the OneStep amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **For each RNA sample prepare a reaction mix according to the table below: Include sufficient reactions for positive and negative controls.**

Component	Volume
oasig® OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix	10 µl
EBOV_S or EBOV_Z-v2.0 primer/probe mix (**BROWN**)	1 µl
Internal extraction control primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (WHITE)	3 µl
Final Volume	**15 µl**

2. **For each RNA sample prepare an endogenous control reaction according to the table below (optional):**
   This control reaction will provide useful information regarding the quality of the biological sample.

Component	Volume
Oasig® OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix	10 µl
Endogenous control primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

3. **Pipette 15µl of these mixes into each well according to your qPCR experimental plate set up.**

4. **Pipette 5µl of RNA template into each well, according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

5. **If a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:**

Component	Volume
Oasig® OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix	10 µl
EBOV_S or EBOV_Z-v2.0 primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (**WHITE**)	4 µl
Final Volume	**15 µl**

6. **Preparation of standard curve dilution series.**

   1) Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
   2) Pipette 10µl of Positive Control Template (RED) into tube 2
   3) Vortex thoroughly
   4) Change pipette tip and pipette 10µl from tube 2 into tube 3
   5) Vortex thoroughly
   Repeat steps 4 and 5 to complete the dilution series

Standard Curve	Copy Number
Tube 1 Positive control (**RED**)	2 x 10^5 per µl
Tube 2	2 x 10^4 per µl
Tube 3	2 x 10^3 per µl
Tube 4	2 x 10^2 per µl
Tube 5	20 per µl
Tube 6	2 per µl

7. **Pipette 5µl of standard template into each well for the standard curve according to your plate set-up**
   The final volume in each well is 20µl.

OneStep RT-qPCR Amplification Protocol

Amplification conditions using oasig™ OneStep or Precision®PLUS OneStep 2X RT-qPCR Master Mix.

	Step	Time	Temp
	Reverse Transcription	10 min	55 °C
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

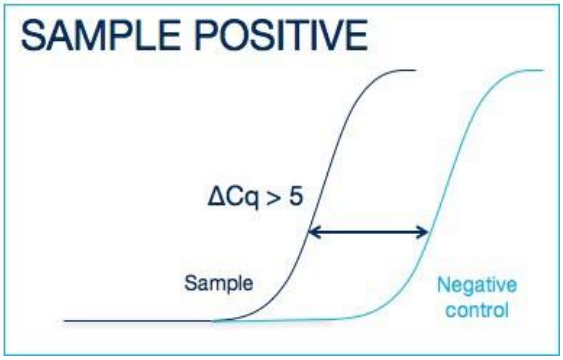
* Fluorogenic data should be collected during this step through the FAM and VIC channels

Interpretation of results

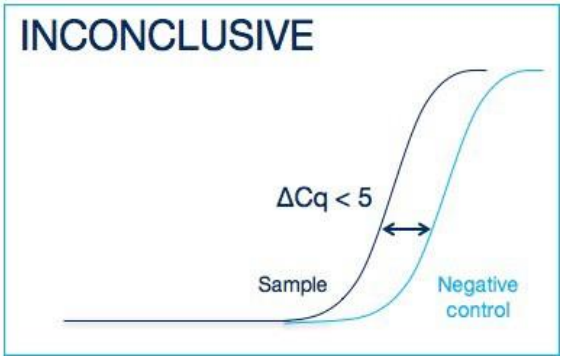
Target (FAM)	Internal control (VIC)	Positive control	Negative control	Interpretation
≤ 30	**+ / -**	**+**	**-**	**POSITIVE QUANTITATIVE RESULT** calculate copy number
> 30	**+**	**+**	**-**	**POSITIVE QUANTITATIVE RESULT** calculate copy number
> 30	**-**	**+**	**-**	**POSITIVE QUALITATIVE RESULT** do not report copy number as this may be due to poor sample extraction
-	**+**	**+**	**-**	**NEGATIVE RESULT**
+ / -	**+ / -**	**+**	**≤ 35**	**EXPERIMENT FAILED due to test contamination**
+ / -	**+ / -**	**+**	**> 35**	*****
-	**-**	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**-**	**+ / -**	**EXPERIMENT FAILED**

Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Internal PCR control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of RNA added to the RT and PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying an target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Endogenous control

The signal obtained from the endogenous control primer and probe set will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.