

Quantification of Sudan Ebola Virus

Nucleoprotein (np) gene

For general laboratory and research use only

Standard kit

150 tests

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Introduction to Sudan 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 five distinct species: Bundibugyo, Ivory Coast, Reston, Sudan and Zaire. 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. Sudan Ebola virus was identified in 1976 and was initially believed to be identical to the Zaire species. It was detected among cotton factory workers in Nzara, Sudan, with the first case reported as a worker exposed to a potential natural reservoir. The virus was not found in any of the local animals and insects that were tested in response.

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 transmission, 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 also 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 hemorrhagic fever, there can be damage to blood vessels and extensive internal and external bleeding or haemorrhage. There is no cure for Ebola haemorrhagic fever, no established drug therapy to treat Ebola infection, and no vaccine that can protect humans against Ebola.

Specificity

The PrimerDesign™ genesig Kit for Sudan Ebola Virus (SUDV) Genomes is designed for the in vitro quantification of SUDV genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the SUDV genome.

The primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

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

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Kit Contents

- SUDV specific primer/probe mix (150 reactions **BROWN**)
FAM labeled, BHQ quenched
- SUDV positive control template (for Standard curve **RED**)
- SUDV RT primer mix (150 reactions **GREEN**)
Required for two step protocol only
- RNase/DNase free water

Reagents and equipment to be supplied by the user

Real-Time PCR Instrument

RNA extraction kit

This kit is designed to work well with all processes that yield high quality RNA with minimal PCR inhibitors.

oasig™ Lyophilised OneStep qRT-PCR MasterMix kit

Contains complete one step qRT-PCR MasterMix

Optional - Reverse Transcription kit and Mastermix

Although a one step RT-PCR protocol is recommended, this kit is designed to work well with a two step protocol. We recommend the use of PrimerDesign Precision™ Reverse Transcription kits and the use of PrimerDesign 2x Precision™ MasterMix for the two step protocol.

Pipettors and Tips

Vortex and centrifuge

Thin walled 1.5 ml PCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20°C on arrival. PrimerDesign does not recommend using the kit after the expiry date stated on the pack. Once the lyophilized components have been re-suspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

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/DNA 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 PrimerDesign genesig detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

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 PrimerDesign pathogen 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. "Black Hole Quencher", "BHQ", "CAL Fluor", "Quasar" and "Pulsar" are registered trademarks of Biosearch Technologies, Inc., Novato, CA. This technology is protected by U.S. and World-wide patents either issued or in application and is licensed and sold under agreement with Biosearch Technologies, Inc. These products are sold exclusively for R&D use by the purchaser. They may not be used for human or veterinary in vitro diagnostic (IVD) applications and they may not be re-sold, distributed or re-packaged without express written authorization from Biosearch Technologies Inc. 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 Applied Biosystems 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. The purchase of Biosearch Technologies products does not, either expressly or by implication, provide a license to use this or other patented technology. Licensing information can be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404 or the Licensing Department at Roche Molecular Systems Inc., 1145 Atlantic Avenue, Alameda, CA 94501."

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Principles of the test

Real-time PCR

A SUDV specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the SUDV DNA/cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled 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 real-time PCR platforms.

One Step vs. Two step real-time PCR

When detecting/quantifying the presence of a target with an RNA genome PrimerDesign recommend the use of a one step qRT-PCR protocol. One step qRT-PCR combines the reverse transcription and real-time PCR reaction in a simple closed tube protocol. This saves significant bench time but also reduces errors. The sensitivity of a one step protocol is also greater than a two step because the entire biological sample is available to the PCR without dilution. This kit will also work well with a two step approach (Precision™ nanoScript reverse transcription kit plus Precision™ MasterMix) if required but the use Precision OneStep™ MasterMix is the preferred method.

Positive control

For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of SUDV copy number / CT value. Alternatively the positive control 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 SUDV gene 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 control does 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 well.

Negative control

To confirm the absence of contamination, 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. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources of contamination should first be explored and removed.

Carry-over prevention using UNG (optional)

Carry over contamination between PCR reactions can be prevented by including uracil-N-glycosylase (UNG) in the reaction mix. Some commercial mastermix preparations contain UNG or alternatively it can be added as a separate component. UNG can only prevent carry over from PCR reactions that include deoxyuridine triphosphate (dUTP) in the original PCR reaction. PrimerDesign recommend the application of 0.2U UNG per assay with a 15 minute incubation step at 37°C prior to amplification. The heat-labile UNG is then inactivated during the Taq polymerase activation step (95°C for 10 minutes).

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Reconstitution 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 lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.
- 2. Reconstitute the kit components in the RNase/DNase-free water supplied, according to the table below:**
To ensure complete resuspension, vortex each tube thoroughly.

Component	Volume
Pre-PCR transparent envelope	
SUDV Primer/Probe mix (BROWN)	165 μ l
SUDV RT primer mix (BROWN)	165 μ l
Post-PCR heat-sealed foil	
Positive Control Template (RED) *	500 μ l

* 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.

One Step RT-PCR detection protocol

A one step approach combining the reverse transcription and amplification in a single closed tube is the preferred method. If, however, a two step approach is required see page 11. The protocols below are for the oasig™ Lyophilised OneStep qRT-PCR MasterMix kit. If you are using an alternative One Step product then follow the manufacturer's instructions.

For optimum performance and sensitivity.

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

For each RNA sample prepare a reaction mix according to the table below:

Component	Volume
oasig™ OneStep qRT-PCR MasterMix	10 µl
SUDV Primer/Probe mix (BROWN)	1 µl
RNA sample	X µl
RNAse/DNAse free water (WHITE)	X µl
Final Volume	20 µl

Standard curve protocol

- I. For each standard curve sample prepare a reaction mix according to the table below

Component	Volume
oasig™ OneStep qRT-PCR MasterMix	10 µl
SUDV Primer/Probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	15 µl

2. Preparation of standard curve dilution series.

- 1) Pipette 900µl of RNAse/DNAse free water into 5 tubes and label 2-6
- 2) Pipette 100µl of Positive Control Template (RED) into tube 2
- 3) Vortex thoroughly
- 4) Change pipette tip and pipette 100µl 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)	2×10^5 per µl
Tube 2	2×10^4 per µl
Tube 3	2×10^3 per µl
Tube 4	2×10^2 per µl
Tube 5	20 per µl
Tube 6	2 per µl

3. 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.

One Step Amplification Protocol

Amplification conditions using oasis™ OneStep 2 × qRT-PCR MasterMix.

	Step	Time	Temp
50 Cycles	Reverse Transcription	10 mins	42 °C
	Enzyme activation	2 mins	95 °C
	Denaturation	10 secs	95 °C
	DATA COLLECTION *	60 secs	60 °C

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

Alternative two step reverse transcription/real-time PCR protocol

Reverse Transcription

If you need to perform separate reverse transcription and amplification (two step Real Time PCR) then we recommend the PrimerDesign Precision nanoScript™ Reverse Transcription kit. A reverse transcription primer (**GREEN**) is included and is designed for use with the Precision nanoScript™ reverse transcription kit. A protocol for this product is available at www.primerdesign.co.uk

1. After reverse transcription, prepare a reaction mix according to the table below for each cDNA sample

Component	Volume
2 x Precision™ MasterMix	10 µl
SUDV Primer/Probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	3 µl
Final Volume	15 µl

2. Pipette 15µl of this mix into each well according to your real-time PCR experimental plate set up.
3. Prepare sample cDNA templates for each of your samples (suggested concentration (5ng/µl) in RNAse/DNAse free water.
If the concentration of RNA that was used to make the cDNA is not known, then dilute your RT reaction mix 1:5 (10µl of sample cDNA and 40µl of water).
4. Pipette 5µl of cDNA template into each well, according to your experimental plate setup. The final volume in each well is 20µl. For negative control wells use 5µl of RNAse/DNAse free water.

Amplification Protocol

Amplification conditions using PrimerDesign 2 X Precision™ MasterMix.

	Step	Time	Temp
50 Cycles	Enzyme activation	10 mins	95 °C
	Denaturation	10s	95 °C
	DATA COLLECTION *	60s	60 °C

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

Interpretation of Results

Target	Negative control	Positive control	Interpretation
+ive	-ive	+ive	+ive
-ive	-ive	+ive	-ive
-ive	-ive	-ive	Experiment fail
+ive	+ive	+ive	Experiment fail

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