Primer Design Ltd

R00966

Ebola Virus:

Sudan and Zaire Strains

Kit version: 1

Target region for both strains:

Nucleoprotein (NP) gene

genesig® Easy Kit

for use on the genesig® q16

50 reactions

GENESIG

Kits by Primerdesign

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

For general laboratory and research use only

genesig® Easy: at a glance guide

For each RNA test

Component	Volume	Lab-in-a-box pipette	
EBOV_S or EBOV_Z-v2.0 primer/probe mix	5 µl		
Your RNA sample	5 µl		
Oasig® OneStep Master Mix	10 µl		

For each positive control

Component	Volume	Lab-in-a-box pipette	
EBOV_S or EBOV_Z-v2.0 primer/probe mix	5 µl		
EBOV_S or EBOV_Z-v2.0 Positive control template	5 µl		
oasig® OneStep Master Mix	10 µl		

For each negative control

Component	Volume	Lab-in-a-box pipette	
EBOV_S or EBOV_Z-v2.0 primer/probe mix	5 µl		
RNase/DNase free water	5 µl		
oasig® OneStep Master Mix	10 µl		

Kit Contents

- **EBOV_S primer/probe mix (BROWN lid)**
- **EBOV_Z-v2.0 primer/probe mix (BROWN lid)**

Once resuspended, the kits should remain at -20°C until ready to use.

- **2x Lyophilised oasig® OneStep Master Mix**

- **2x oasig® resuspension buffer (BLUE lid)**

- **EBOV_S positive control template (RED lid)**
- **EBOV_Z-v2.0 positive control template (RED lid)**
- **Internal extraction control RNA template (BLUE lid)**

- **RNase/DNase-free water (WHITE lid)**

- **2x Template preparation buffer (YELLOW lid)**

- **2x 54 genesig®q16 reaction tubes**

Reagents and equipment to be supplied by the user

genesig® q16 instrument

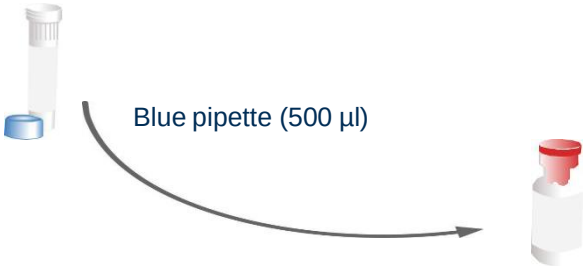
genesig® Easy Extraction Kit
This kit is designed to work well with all processes that yield high-quality RNA and DNA, but the genesig® Easy extraction method is recommended for ease of use.

genesig® Lab-In-A-Box
The genesig® Lab-In-A-Box contains all of the pipettes, tips and racks that you will need to use a genesig® Easy kit. Alternatively, if you already have these components and equipment, these can be used instead.

Step-by-step guide

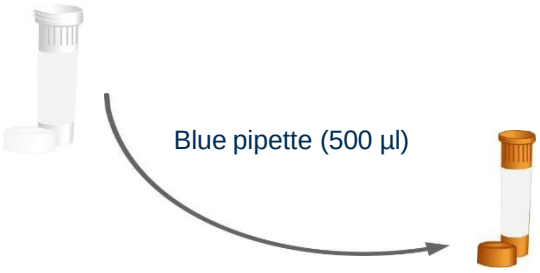
1. Resuspend the test components

Complete this step for each primer/probe mix.



Use the blue pipette to transfer 500 µl* of the oasig® OneStep Master Mix resuspension buffer into the tube of lyophilised oasig® OneStep Master Mix and mix well by gently swirling.

*Transferring 525 µl of the oasig® resuspension buffer to your oasig® OneStep Master Mix (instead of the 500 µl recommended above) will enable you to take full advantage of the 50 reactions by accounting for volume losses during pipetting. In order to do so with the genesig® Easy fixed volume pipettes use 1x blue, 2x red and 1x grey pipettes to make the total volume. Please be assured that this will not adversely affect the efficiency of the test.



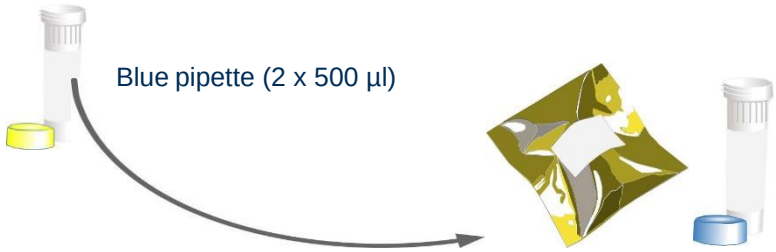
Then use the blue pipette to transfer 500 µl of water into one of the brown tubes labelled EBOV_S or EBOV_Z-v2.0 primers/probe. Cap and shake tube to mix. A thorough shake is essential to ensure that all components are resuspended. **Failure to mix well can produce poor kit performance.**

These components are now ready to use. Store them in the freezer from hereon.

Top tip

- Ensure that the primer/probe mixes are mixed thoroughly before each use by shaking and pipetting up and down 10 times.
- Once resuspended do not expose genesig® Easy kit to temperatures above -20°C for longer than 30 minutes at a time.

2. Internal extraction control



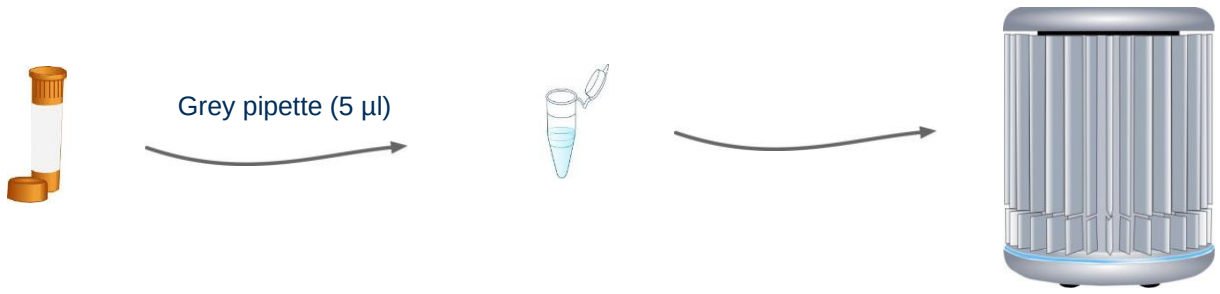
Use the blue pipette to transfer 1000 µl (2 x 500 µl) of template preparation buffer into the Internal Extraction Control RNA template tube. Cap and shake tube to mix.

Your kit contains Internal Extraction Control RNA template. This is added to your biological sample at the beginning of the RNA extraction process. It is extracted along with the RNA from your target of interest. The q16 will detect the presence of this Internal Extraction Control RNA template at the same time as your target. This is the ideal way to show that your RNA extraction process has been successful.

If you are using an alternative extraction kit:
Use the red pipette to transfer 10 µl of Internal Extraction Control RNA template to your sample **after** the lysis buffer has been added, then follow the rest of the extraction protocol.

If using samples that have already been extracted:
Use the grey pipette to transfer 5 µl of Internal Extraction Control RNA template to your extracted sample.

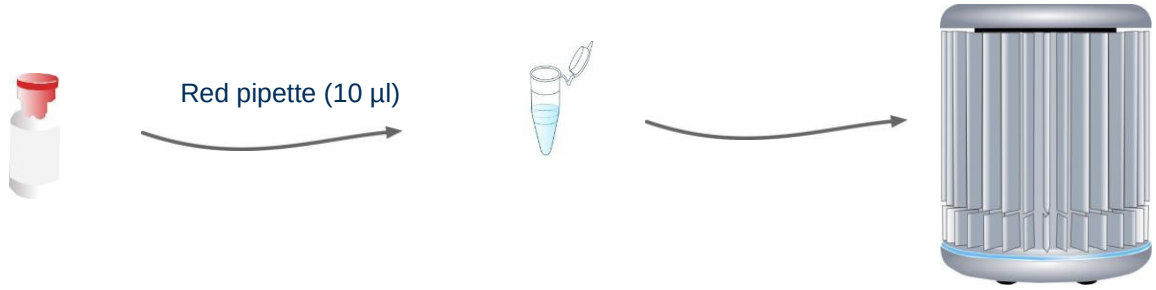
3. Add primer/probe mix to all reaction tubes



For every reaction run, use the grey pipette to add 5 µl of your EBOV_S or EBOV_Z-v2.0 primers/probe mix to every tube.

Top tip
• Always pipette the primer/probe mix directly into the bottom of the tube. • You can label the tube lids to aid your reaction set-up but avoid labelling tube sides.

4. Add Master Mix to all reaction tubes



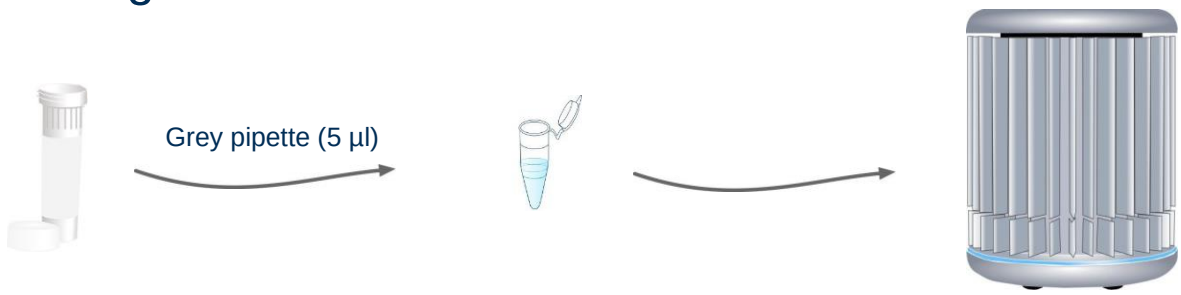
For every reaction run, use the red pipette to add 10 µl of the Oasig® OneStep Master Mix to the tubes containing primer/probe mix.

Move swiftly to begin your q16 run, as any delay after the Oasig® OneStep Master Mix has been added can affect the sensitivity of your test.

Top tip

- Always add the oasig™ OneStep Master Mix to the side of the tube to reduce the introduction of bubbles.

5. Negative control



For each test, you will require a negative control. Instead of RNA, water is used. This sample should typically prove negative, thus proving that all of your positive samples are positive.

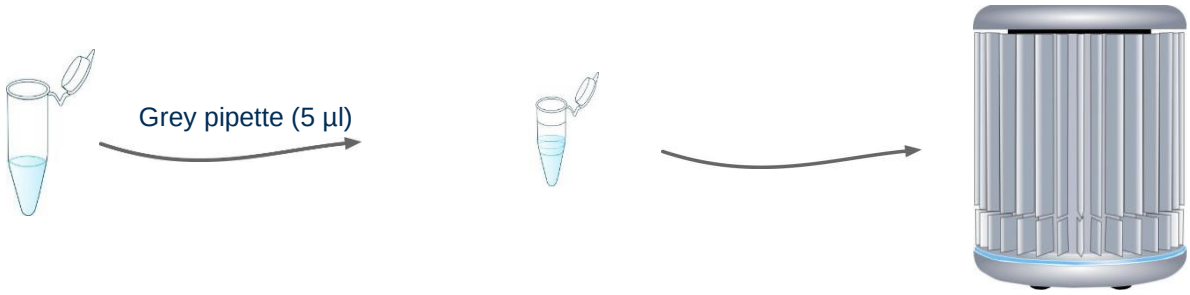
To create a negative control reaction, simply use the grey pipette to add 5 µl of the water to the required reaction tubes. Close these tubes after adding the water.

Because some genesig® kit targets are common in the environment, you may occasionally see a “late” signal in the negative control. The q16 software will take this into account accordingly.

Top tip

- Always add the water to the side of the tube to reduce the introduction of bubbles.

6. Set up a test

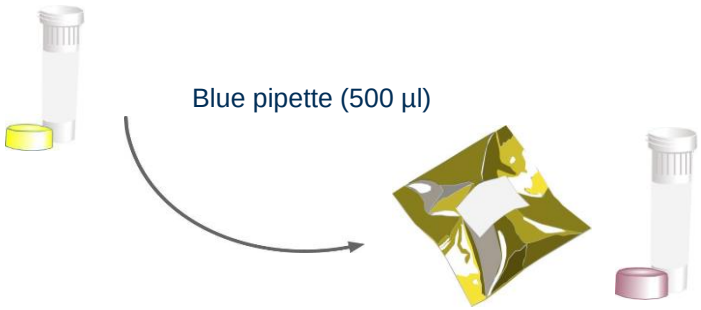


For each sample, you wish to analyse, use the grey pipette to add 5 µl of your RNA sample to the required reaction tubes. Close these tubes after adding the sample. Always change pipette tips between samples.

Top tip

- Always add the RNA sample to the side of the tube to reduce the introduction of bubbles.

7. Positive control

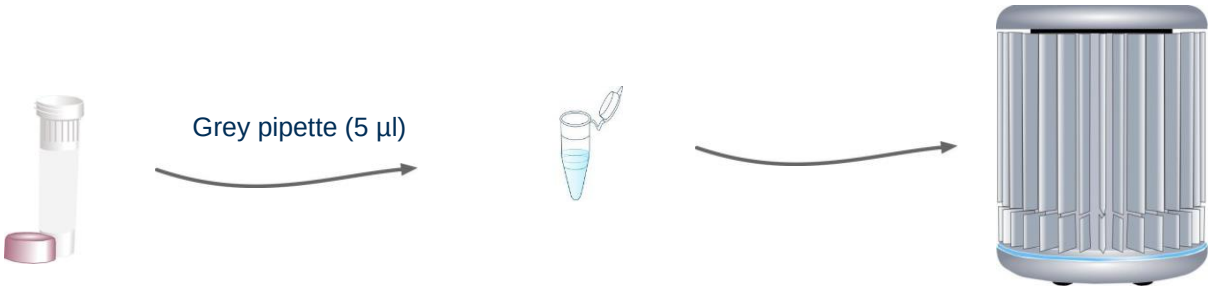


Use the blue pipette to transfer 500 µl of template preparation buffer into the positive control template tube. Cap and shake tube to mix.

Each time you run a test, you will require a positive control. It serves two purposes:

1. It will always test positive to show that everything is working as it should be.
2. The q16 software knows how much RNA is present in the positive control. So, it can automatically compare your sample of interest with the positive control to calculate the amount of target RNA in your sample.

To create a positive control reaction, simply use 5µl of the positive control template instead of your RNA sample.



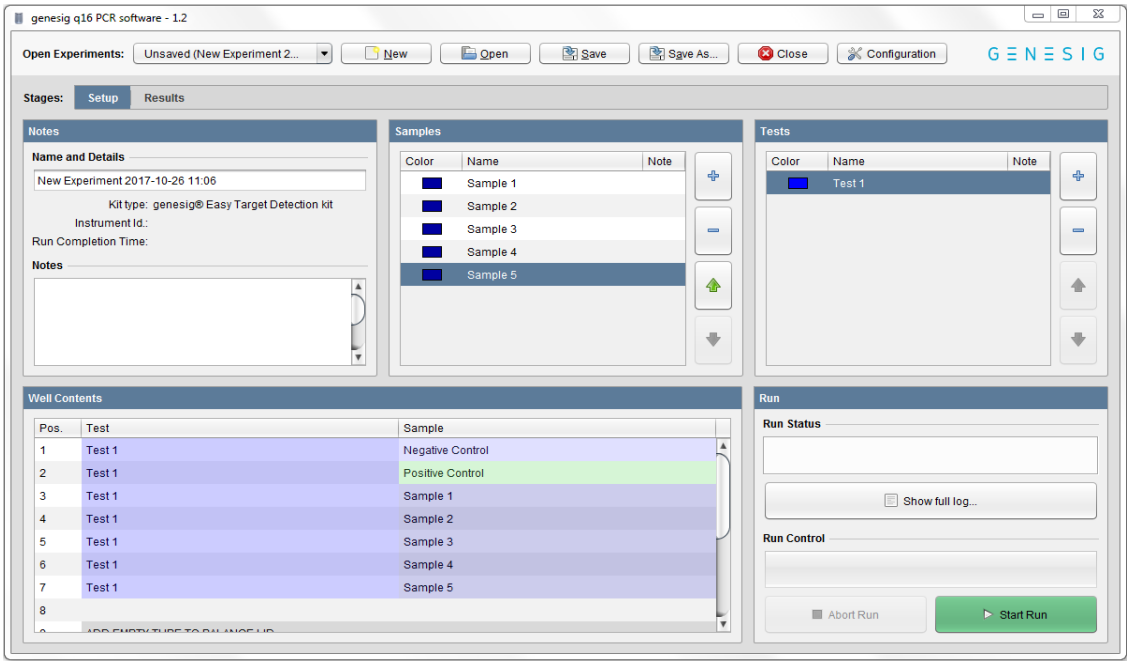
Take great care when setting up your positive control. The positive control template has the potential to give you a false positive signal in your other samples. Set up the positive controls last after all other sample tubes are closed. Always change pipette tips between samples. You may even choose to set up positive controls in a separate room.

Top tip

- Always add the positive control template to the side of the tube to reduce the introduction of bubbles.

8. Running the test

Place the tubes into the correct positions in your q16 as defined by the software; this may include positioning empty tubes to ensure that the q16 lid is balanced. The run can then be started.



Top tip

- Before loading tubes into the q16, check for bubbles! Flick the bottom of the tubes to remove any bubbles that may have formed during the test set-up.
- Apply centrifugal force with a sharp wrist action to ensure all solution is at the bottom of the reaction tube.
- When repeating a test, you can use a previous file as a template by clicking ‘open’ then selecting File name > Files of Type > Experiment file as template.

What do my results mean?

Analysis of your data is carried out automatically by the q16. The following information is designed to help you fully understand a result or to troubleshoot:

“Positive”

Explanation
Your sample has produced a positive result. Your target of interest is present, and you can use the reported quantity.

“Negative”

Explanation
Your sample has produced a negative result. The target is not present in your sample.

“Test contaminated”

Explanation
The Negative Control should be completely free of any DNA/RNA. If you see this error message, it means that at some point during the set-up, the Negative Control has been contaminated with DNA/RNA and has given a positive signal. This contamination has invalidated the test. The Positive Control and your test samples are both possible sources of contaminating DNA/RNA. The genesig® q16 reaction tubes from previous runs will also contain very high amounts of DNA, so it is important that these are carefully disposed of after the run is completed and NEVER OPENED. It may be the case that your kits have become contaminated, which will lead to the same problem occurring repeatedly.

Solutions

1. Clean your working area using a commercial DNA remover solution to ensure the area is
2. DNA-free at the start of your run and re-run the test.
3. If the problem persists, then the kit has become contaminated, and it will have to be discarded and replaced with a new kit. When you open the new kit, run a simple test to show that changing the kit has solved the problem. Prepare a test that includes only the Positive Control, the Negative Control and one ‘mock sample’. For the ‘mock sample’ add water instead of any sample RNA. The result for the Negative Control and the mock sample should be negative, indicating that contamination is no longer present.

Preventive action
An ideal lab set-up has a ‘Clean area’ where the test reagents are prepared and a ‘sample area’ where DNA/RNA samples and the Positive Control template are handled. The best workflow involves setting up all the test components (excluding the positive control template) in the clean area and then moving the tests to the sample area for the sample and Positive Control addition.

“Sample preparation failed”

Explanation
The test has failed because the quality of the sample was not high enough. The Internal Extraction Control component identifies whether the sample has been prepared correctly and is of suitable quality. This error message means that this quality control test has failed, and the sample quality is not high enough for analysis.

Solutions
1. Check the sample preparation protocol for any user errors, then repeat.
2. Poor quality samples can result from overloading the sample preparation protocol with too much starting material. Try reducing the amount of starting material then repeat.
3. Failing to add the Internal Extraction Control RNA template to your sample during the sample preparation protocol can also lead to a reported result of “sample preparation failed”. Ensure that this step has not been overlooked or forgotten. If your samples are derived from an archive store or from a process separate from your genesig® Easy extraction kit; you must add 5µl of Internal Extraction Control RNA template into each 0.5 ml of your sample to make it suitable for use on the q16.

“Positive result, poor quality sample”

Explanation
The test is positive, so if you are only interested in obtaining a ‘present or absent’ answer for your sample, then your result is reliable. However, the test contains an Internal Extraction Control component that identifies if the sample is of high quality. This quality control test has failed, therefore the sample is not of high enough quality to accurately calculate the exact copy number of RNA present. If you require quantitative information for your sample, then proceed with the solutions below.

“Test failed”

Explanation
The test has failed because the Positive Control has not worked. The Positive Control is present to show that all aspects of the test are working correctly together. When this control test fails, the test as a whole is invalidated. This finding indicates that a problem has occurred in the reaction set-up part of the experiment and has nothing to do with sample preparation.

Solutions
1. Check the entire workflow and test set-up to look for any user errors, then repeat the test e.g., have the right colour pipettes and solutions been used with the correct tubes?
2. Ensure the positive and negative controls are inserted into the correct wells of your q16.
3. A component of the test may have ‘gone off’ due to handing errors, incorrect storage or exceeding the shelf life. When you open a new kit, run a simple test to show that changing the kit has solved the problem. Prepare a test that includes only the Positive Control, the Negative Control and one ‘mock sample’. For the ‘mock sample’ add internal control template instead of any sample RNA. If the Positive Control works, the mock sample will now be called as a negative result.

“Test failed and is contaminated”

Explanation
The Positive Control is indicating test failure, and the Negative Control is indicating test contamination. Please read the “Test Failed” and “Test contamination” sections of this technical support handbook for a further explanation.

Solution
1. For appropriate solutions, read both the “Test failed” and “Test contaminated” sections of this handbook.

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®Easy 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 storage and stability

This lyophilised 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.
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/DNA integrity.

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.

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 US Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by US Patent 5,538,848, owned by The Perkin-Elmer Corporation.

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