Primerdesign™ Ltd

Rift Valley Fever Virus

genesig® Easy Kit

for use on the genesig® q16

50 reaction

GENESIG

Kits by Primerdesign

For general laboratory and research use only

genesig® Easy: at a glance guide

For each RNA test

Component	Volume	Lab-in-a-box pipette	
RVFV 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	
RVFV primer/probe mix	5 µl		
Positive control template	5 µl		
oasig OneStep Master Mix	10 µl		

For each negative control

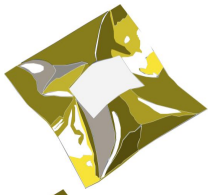
Component	Volume	Lab-in-a-box pipette	
RVFV primer/probe mix	5 µl		
Water	5 µl		
oasig OneStep Master Mix	10 µl		

Kit Contents

- **RVFV specific primer/probe mix (BROWN)**
  Once resuspended the kits should remain at -20ºC until ready to use.

- **Lyophilised oasig™ OneStep Master Mix**

- **Lyophilised oasig™ OneStep Master Mix resuspension buffer (BLUE lid)**



- **RVFV positive control template (RED lid)**

- **Internal extraction control RNA (BLUE lid)**

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

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

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

Reagents and equipment to be supplied by the user

genesig® q16 instrument

genesig® Easy Extraction Kit

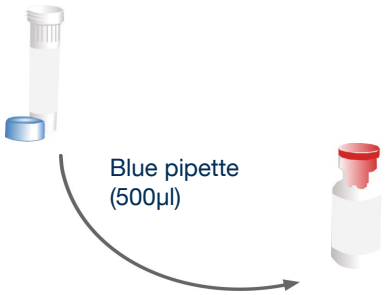
This kit is designed to work well with all processes that yield high quality RNA and DNA but the genesig Easy extraction method is recommended for ease of use.

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.

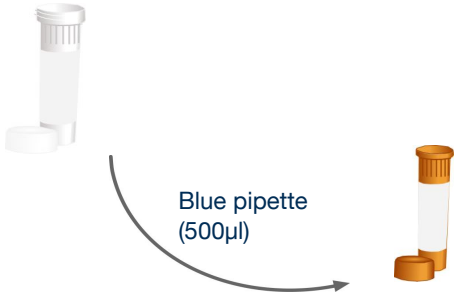
Step-by-step guide

1. Resuspend the test components



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

*Transfering 525µl of the oasig OneStep Master Mix resuspension buffer to your oasig OneStep Master Mix (instead of the 500µl 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 500µl of water into the brown tube labelled RVFV 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.**

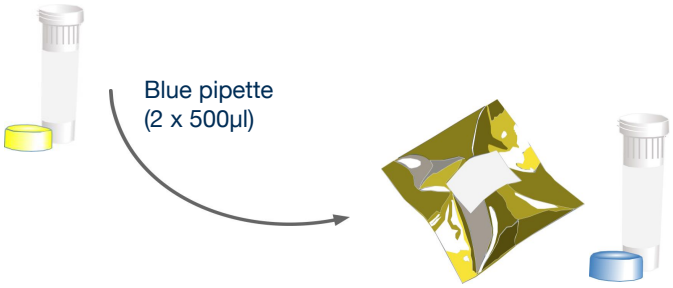
These components are now ready to use.

Store them in the freezer from hereon.

Top tip

- Ensure that the primer/probe mix is mixed thoroughly before each use by shaking.
- 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 1000µl (2 x 500µl) of template preparation buffer into the Internal Extraction Control RNA tube. Cap and shake tube to mix.

Your kit contains Internal Extraction Control RNA. 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 at the same time as your target. This is the ideal way to show that your RNA extraction process has been successful.

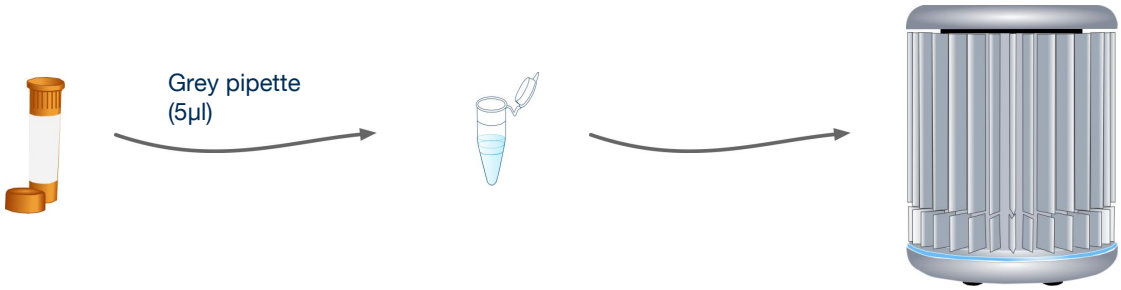
If you are using an alternative extraction kit:

Use the red pipette to transfer 10µl of Internal Extraction Control RNA 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 5µl of Internal Extraction Control RNA to your extracted sample.

3. Add primer/probe mix to all reaction tubes

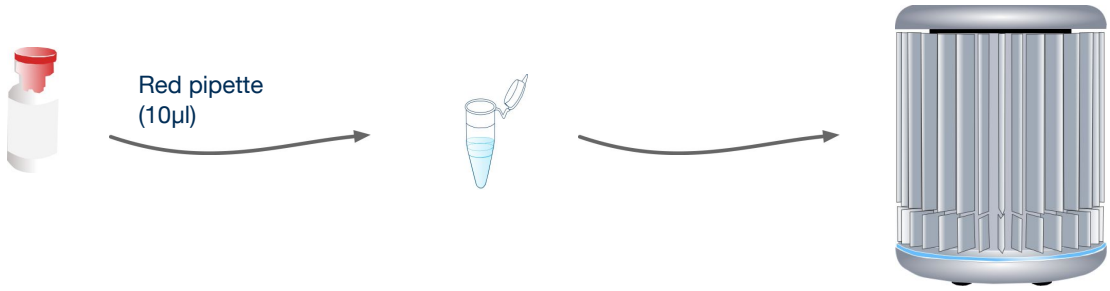


For every reaction to be run, use the grey pipette to add 5µl of your RVFV primers/probe mix to every tube.

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 setup but avoid labelling tube sides.

4. Add Master Mix to all reaction tubes



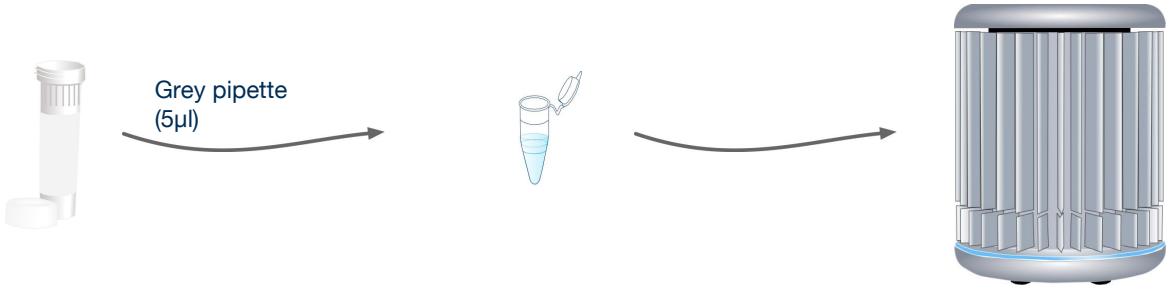
For every reaction to be run, use the red pipette to add 10µl of the oasig OneStep Master Mix to the tubes containing primer/probe mix.

Move swiftly to begin your q16 run, as any delay after the oasig OneStep Master Mix has been added can effect 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 really are positive.

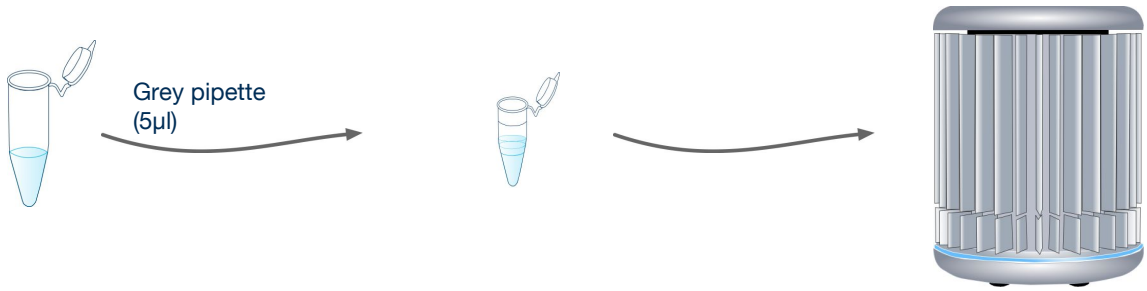
To create a negative control reaction simply use the grey pipette to add 5µl of the water to the required reaction tubes. Close these tubes after adding the water.

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.

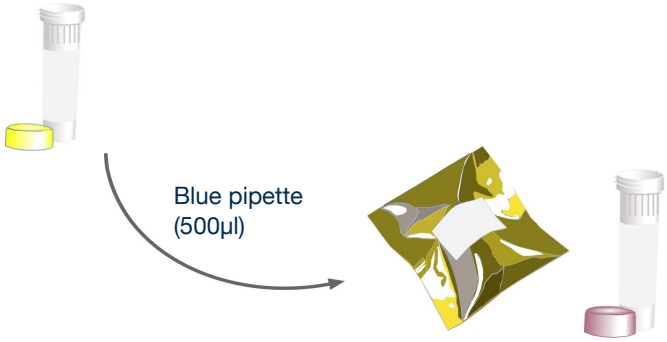
6. Set up a test



For each sample you wish to analyse, use the grey pipette to add 5µl 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

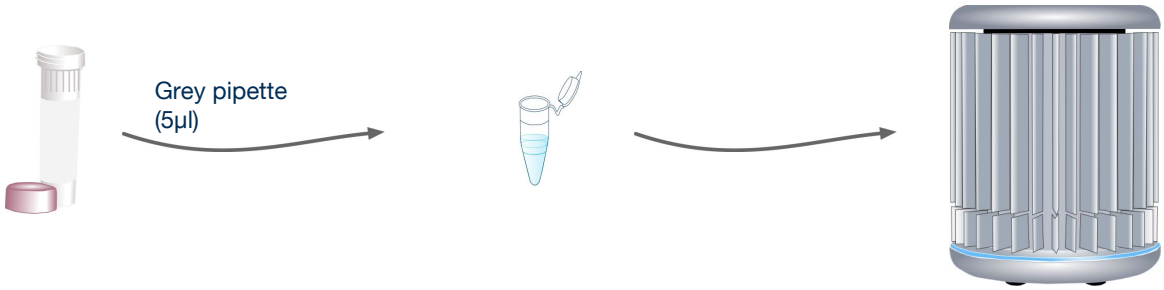


Use the blue pipette to transfer 500µl of template preparation buffer into the positive control template tube. Cap and shake tube to mix.

Each time you run a test you will require a positive control. This is a small portion of RNA from your target of interest. It serves two purposes:

1. It will always test positive so it shows 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 instead of your RNA sample.



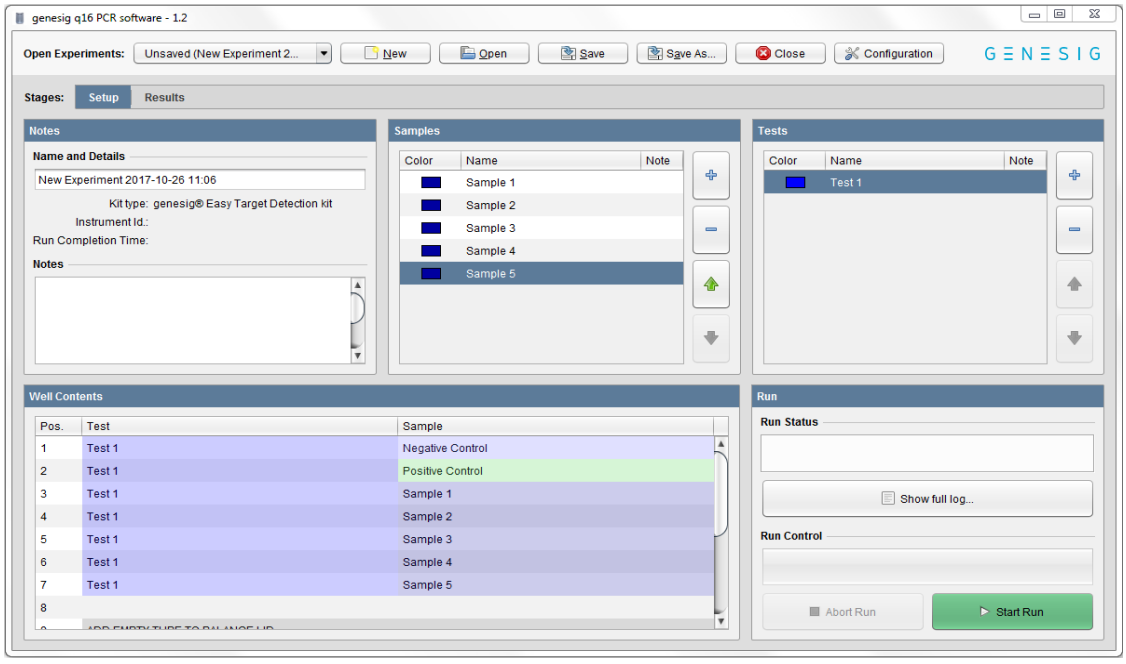
Take great care when setting up your positive control. The positive control template has the potential to give you a false positive signal in your other samples. Set positive controls up last after all other sample tubes are closed. Always change pipette tips between samples. You may even choose to set 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 of 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 setup.
- 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 genesig 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 setup, 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 DNA free at the start of your run and re-run the test.
2. 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 which 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 sample and Positive Control addition. If this method is followed then the kit components are always kept away from possible sources of contamination. For extra security the Negative Control can be completely prepared and sealed in the clean area. All work areas should be decontaminated regularly with DNA remover.

“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 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 into each 0.5ml 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 and the sample is not therefore 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.

Solutions

1. For appropriate solutions, read the “Sample preparation failed” section of this handbook.

“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 which 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.

Rift Valley Fever Virus

Rift Valley fever is an arthropod-borne viral anthropozoonosis caused by a single stranded RNA phlebovirus called Rift Valley fever virus (RVFV). RVFV is transmitted by mosquitoes and causes severe hemorrhagic illness among humans, while in livestock it causes fever and high abortion rates. The genome consists of three negative sense, single stranded RNA segments designated L (large), M (medium) and S (small). The RVFV L segment (6.4 kb) encodes an RNA-dependent RNA polymerase, the M segment (3.9 kb) encodes two structural glycoproteins (G1 and G2) and two proteins of unknown function (NSm and 78-kDa protein), and the S segment (1.7 kb) encodes a nucleoprotein (N) in the anti-viral sense and a non-structural protein (NSs) in the viral sense.

Rift valley fever virus primarily spreads through transcutaneous transmission, aerosol transmission and insect bites. RVF is most commonly associated with mosquito-borne epidemics during years of unusually heavy rainfall. The mosquitoes, usually of the genus Aedus, breed in stagnant ponds that have been created by the heavy rainfall. As the mosquitoes grow in number the epizootic and epidemic cycles can occur. The mosquito eggs are naturally infected with the RVF virus, and the resulting mosquitoes transfer the virus to the livestock on which they feed. Once the livestock is infected, other species of mosquitoes can become infected from the animals and can spread the disease. During dry periods, the virus lies dormant in drought-resistant mosquito eggs over much of the African continent. The virus is also transmitted via contact with the blood, secretions, or excretions of infected animals. Because the virus affects livestock, contact with diseased animals can be via slaughtering or handling of infected animals, herding, touching contaminated meat from infected animals, and so on.

RVFV forms plaques, usually targeting the liver and brain. After a mosquito bite, the virus moves from the skin to draining lymph nodes, where it replicates. Efferent lymphatics spread the virus throughout the body. As the liver is rapidly invaded, hepatocytes become involved. The virus may also cross the blood-brain barrier and infect neurons and glia. Patients suffering from RVF may show no symptoms at all, flu-like symptoms or sudden onset of mild fever with symptoms of liver and kidney disorder. More severe forms of the disease include the ocular, meningoencephalitis and haemorrhagic fever forms. Typically patients recover within 2 to 7 days after onset of illness. Approximately 1% of humans that become infected with RVFV die of the disease. Fatalities are significantly higher for infected livestock.

Specificity

The Primerdesign genesig Kit for Rift Valley Fever Virus (RVFV) genomes is designed for the in vitro quantification of RVFV genomes. The kit is designed to have a broad detection profile. Specifically, the primers represent 100% homology with over 95% of the NCBI database reference sequences available at the time of design.

The dynamics of genetic variation means that new sequence information may become available after the initial design. Primerdesign 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 enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

Kit storage and stability

This lyophilised kit is stable at room temperature but should be stored at -20ºC 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.

Primerdesign 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 genesig RVFV 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 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.

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