Primerdesign™ Ltd

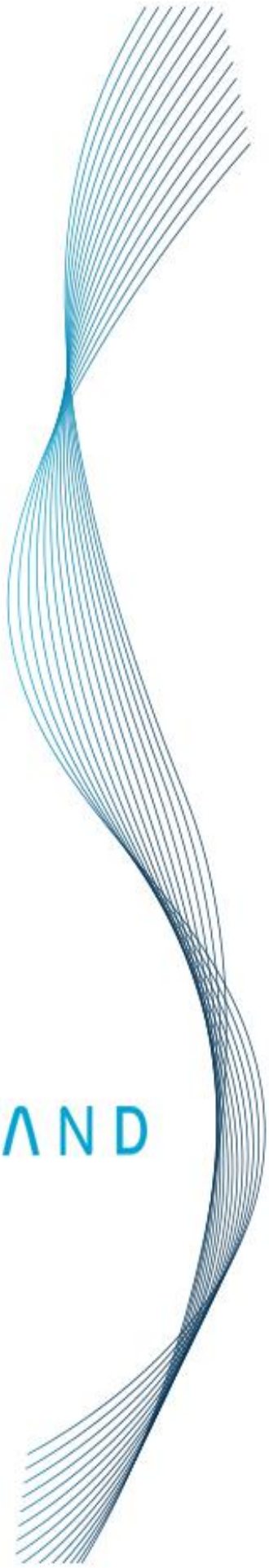
OnDemand

Easy Kit

(Incl. DNA, RNA and Speciation)

Handbook

50 tests



ONDEMAND

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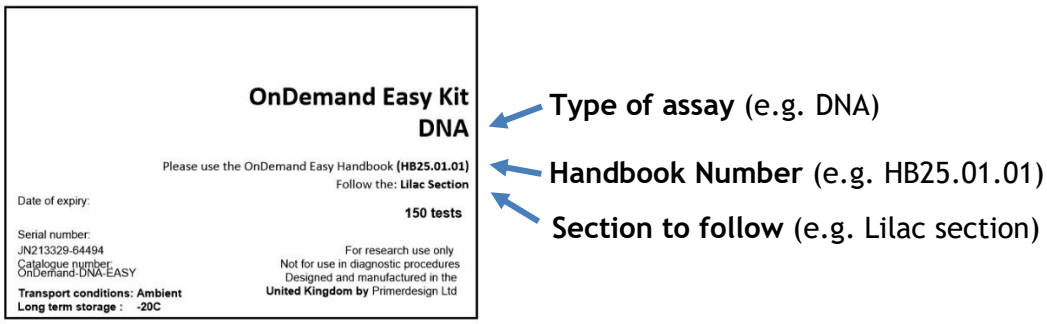
Contents Page:

Where to find the correct section to follow:

Please refer to your assay format for correct handbook procedure to follow. The reference is located on front of the kits packaging material. Below is an example pouch label, the handbook number is referenced, and this number can be found on the footer of the document (HB25.01.01). The section to follow is also referenced via the colour section and type of assay (e.g. DNA).

Example Pouch label:



If not, or not accessible, the correct link should be supplied via the website and/or email correspondence. However, if still unclear or any other question please do not hesitate to contact us via enquiry@primerdesign.co.uk or (+44) 2380 748 830.

Introduction to OnDemand Kits

OnDemand Kits are Real-Time PCR Kits which we have the capability and capacity to design and develop. They are for 'hotspot' target organisms which have particular importance in their individual areas of interest and therefore we understand the importance of developing a Kit. The OnDemand Kits can be developed on request and to meet your specific requirements meaning you will be provided with a Kit which is of the highest quality and which works for you.

We offer specific qPCR kit development including primer/probe design, PCR mix optimization and Kit development to ensure a very effective qPCR reaction. We have already developed a huge range kits as part of our ever expanding genesig catalogue. This service allows for the combination of bioinformatic design and development, in conjunction with laboratory testing and evaluation, to provide personally designed primers to match your exact needs and ensure high levels of sensitivity and specificity.

Specificity

The Primerdesign OnDemand Kits for each specific target are designed for the in vitro detection and/or quantification of the specific target. 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.

Kit storage and stability

This kit is stable at room temperature but must be stored at -20ºC on arrival. Once the Lyophilised components have been resuspended they must not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing must 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.
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 (An internal PCR control is supplied to test for nonspecific 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 OnDemand detection Kits have very high priming efficiencies of >90% and can detect less than 100 copies of target template.

Quality Control

As part of our ISO9001 and ISO13485 certified quality assurance systems, all Primerdesign products are verified to ensure the highest levels of performance and reliability.

Principles of the test

Real-time PCR

The targets specific primer and probe mix is provided, and this can be detected through the specified channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target 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 qPCR platforms.

N.B. RNA Kits only OneStep vs. Two step real-time PCR

When detecting/quantifying the presence of a target with an RNA genome Primerdesign recommend the use of a OneStep RT-qPCR protocol. OneStep RT-qPCR combines the reverse transcription and qPCR reaction in a simple closed tube protocol. This saves significant bench time but also reduces errors. The sensitivity of a OneStep protocol is also greater than a two-step because the entire biological sample is available to the PCR without dilution.

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 target copy number / Cq 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 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 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.

Internal extraction control

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

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

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 Kit 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

Primerdesign™ is a trademark of Primerdesign Ltd.
The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. BI, ABI PRISM® GeneAmp® and MicroAmp® are registered trademarks of the Applera Genomics (Applied Biosystems Corporation). BIOMEK® is a registered trademark of Beckman Instruments, Inc.; iCycler™ is a registered trademark of Bio-Rad Laboratories, Rotor-Gene is a trademark of Corbett Research. LightCycler™ is a registered trademark of the Idaho Technology Inc. GeneAmp®, TaqMan® and AmpliTaqGold® are registered trademarks of Roche Molecular Systems, Inc., The purchase of the Primerdesign ™ reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc

Primerdesign™ Ltd

OnDemand Easy Kit

DNA Kits

50 reaction
for use on the genesig® q16

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Kit Contents

***N.B.** Once resuspended the Kits must remain at **-20ºC** until ready to use.*

Target specific primer/probe mix (BROWN Lid)

Lyophilised oasig™ Master Mix

Lyophilised oasig™ Master Mix resuspension buffer (BLUE lid)

Target positive control template (RED lid)

Internal extraction control DNA (BLUE lid)

RNase/DNase free water (WHITE lid)

Template preparation buffer (YELLOW lid)

54 x genesig® q16 reaction tubes

OnDemand Easy Kit (DNA Kit): at a glance guide

For each Sample DNA test

Component	Volume	Lab-in-a-box pipette	
Target reaction mix	10 µl		
Your DNA sample	10 µl		

For each positive control

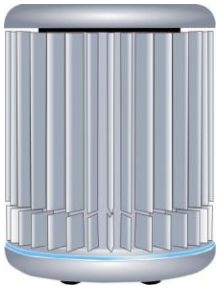
Component	Volume	Lab-in-a-box pipette	
Target reaction mix	10 µl		
Positive control template	10 µl		

For each negative control

Component	Volume	Lab-in-a-box pipette	
Target reaction mix	10 µl		
Water	10 µl		

Reagents and equipment to be supplied by the User

genesig® q16 instrument



The genesig q16 is a revolutionary instrument launched by Primerdesign Ltd. The instrument is designed to accompany the genesig® Easy product range which includes Kits for more than 600 different DNA testing applications. The q16 is designed to make DNA testing affordable and easy for anyone in any business.

genesig® Easy DNA/RNA Extraction Kit

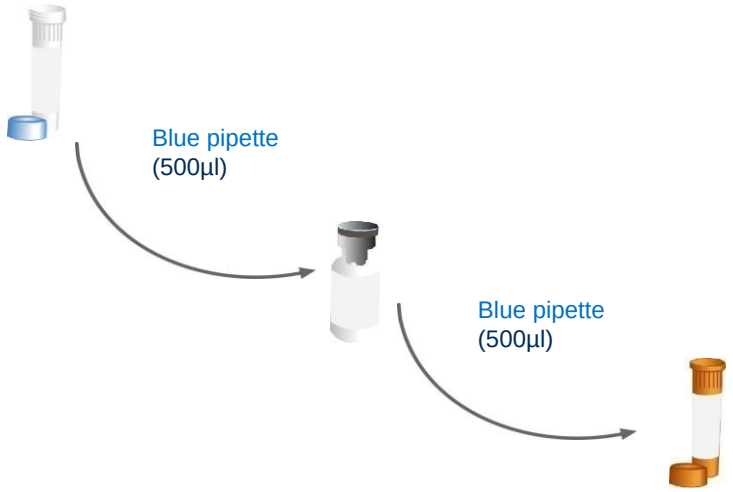
The genesig® Easy DNA/RNA Extraction Kit is designed to work well with all processes that yield high quality DNA but the genesig® Easy extraction method is recommended for ease of use.

genesig® Lab-In-A-Box

The genesig® Lab-In-A-Box contains all 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. Create your reaction mix



Use the blue pipette to

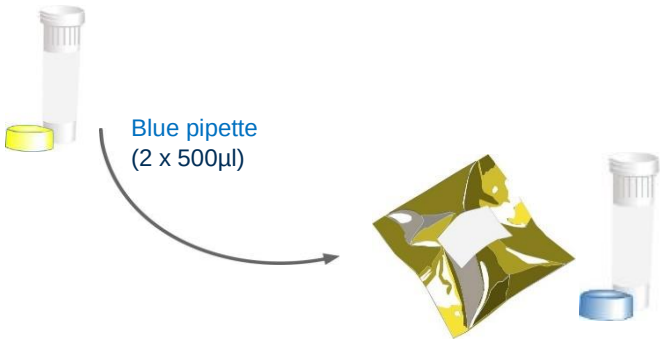
a. Transfer **500 µl*** of the oasig Master Mix resuspension buffer (blue lid) into the tube of Lyophilised oasig Master Mix
b. Mix well by gently swirling (lid on)
c. Then transfer all that master mix into the brown tube labelled Target primers/probe (Brown Lid)
d. Thorough shake is essential to ensure all components are resuspended
e. Leave to stand for **5 minutes**. Now your reaction mix is ready to use
f. Store the reaction mix in the freezer (-20°C) from hereon

Failure to mix well can produce poor Kit performance.

Once resuspended do not expose genesig Easy Kit to temperatures above -20°C for longer than 30 minutes at a time.

*Transferring 525µl of the oasig Master Mix resuspension buffer to your oasig 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.

2. Internal extraction control



a. Use the blue pipette to transfer 1000 µl (2 x 500 µl) of template preparation buffer (yellow Lid) into the Internal Extraction Control DNA tube (blue Lid)

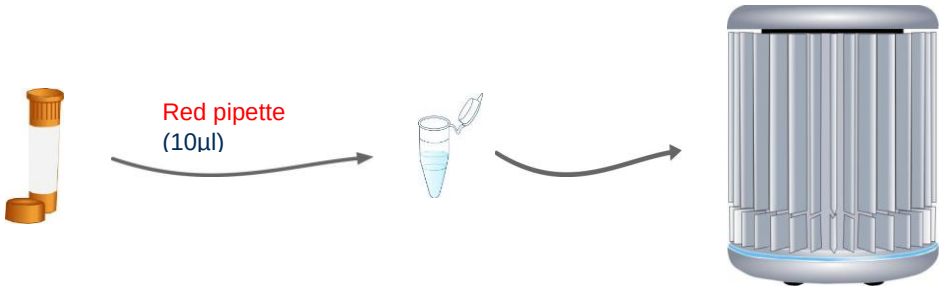
b. Cap and shake tube to mix.

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

OR

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

3. Add reaction mix to all reaction tubes

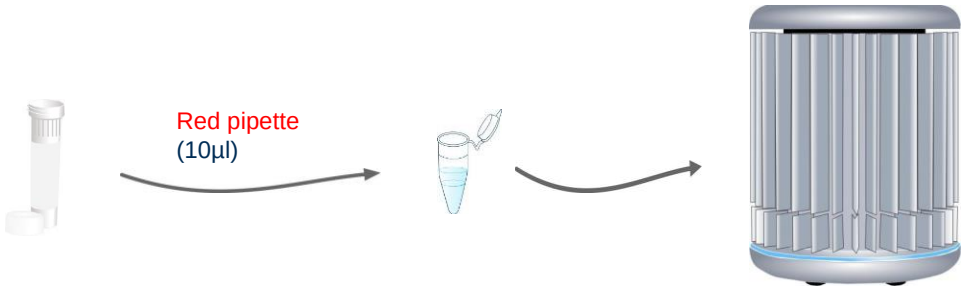


a. For every reaction to be run, use the red pipette to add 10 µl of your Target reaction mix to every tube.

#PRIMERDESIGNTOPTIP

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

4. Negative control



a. To create a negative control reaction simply use the red pipette to add 10 µl of the water to the required reaction tube
b. Close these tubes after adding the water

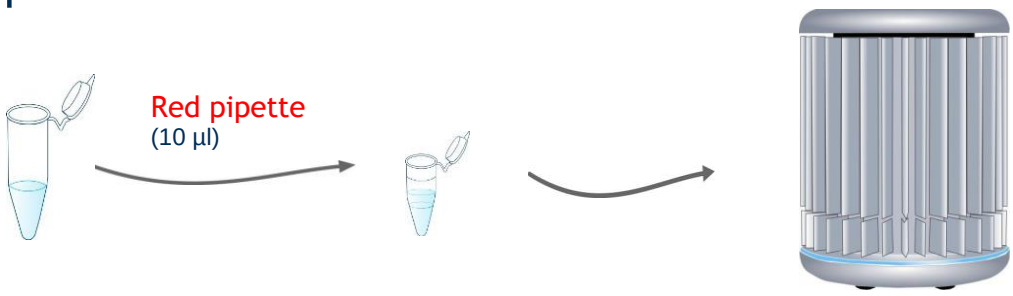
For each test you will require a negative control. Instead of DNA, water is used. This sample should typically prove negative thus proving that all your positive samples really are positive.

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.

#PRIMERDESIGNTOPTIP

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

5. Set up a Test

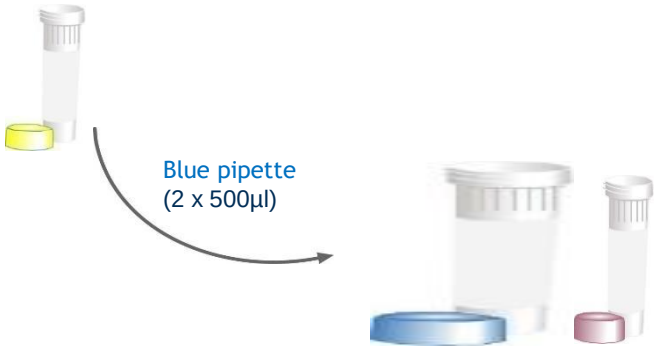


a. For each sample you wish to analyse, use the red pipette to add 10 µl of your DNA sample to the required reaction tubes *(**N.B.** Always change pipette tips between samples)*
b. Close these tubes after adding the sample.

#PRIMERDESIGNTOPTIP

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

6. Positive control



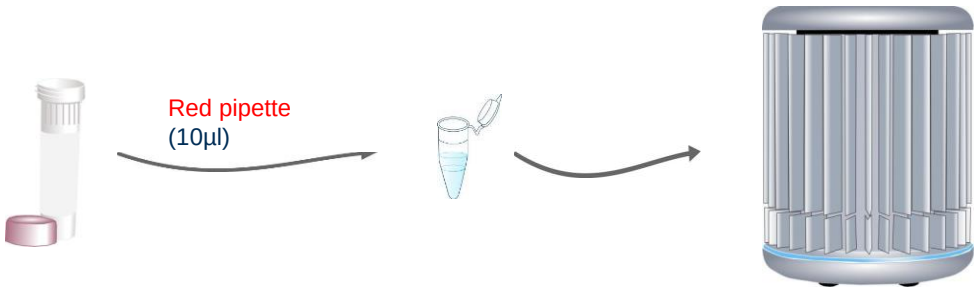
a. Use the blue pipette to transfer 1000 µl (2 x 500 µl) of template preparation buffer (Yellow Lid) into the positive control template tube
b. Cap and shake tube to mix.

Why run a Positive Control?

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

a. It will always test positive, so it shows that everything is working as it should be.
b. The q16 software knows how much DNA 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 DNA in your sample.

c. To create a positive control reaction, simply use Red Pipette (10 µl) of the positive control instead of your DNA sample.

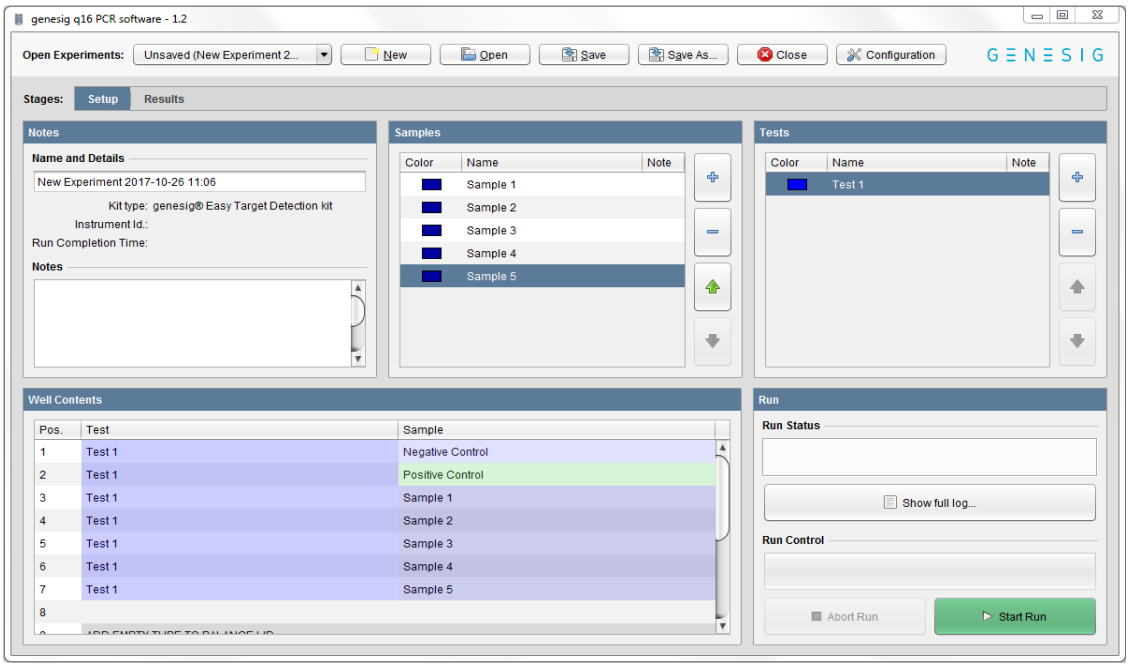


#PRIMERDESIGNTOPTIP

- 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.

7. 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.



#PRIMERDESIGNTOPTIP

- 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

8. What do my results mean?

Analysis of your data is carried out automatically by the genesig q16.

a. 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 result.

OR

"Negative"

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

OR

"Test contaminated"

Explanation
The Negative Control should be completely free of any DNA. If you see this error message it means that at some point during the setup, the Negative Control has been contaminated with DNA 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. 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

a. 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

b. 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.

c. To 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 DNA. The result for the Negative Control and the mock sample should be negative indicating that contamination is no longer present.
d. An ideal lab set-up has a 'Clean area' where the test reagents are prepared and a 'sample area' where DNA samples and the Positive Control template are handled
    i. The best workflow involves setting up all the test components (excluding the positive control template) in the clean area
    ii. 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.
    iii. For extra security the Negative Control can be completely prepared and sealed in the clean area.
    iv. All work areas should be decontaminated regularly with DNA remover.

OR

"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

a. Check the sample preparation protocol for any user errors then repeat.
b. 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.
c. Failing to add the Internal extraction Control DNA 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 DNA into each 0.5ml of your sample to make it suitable for use on the q16.

OR

"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 DNA present. If you require quantitative information for your sample, then proceed with the solutions below.

Solution

For appropriate solutions, read the **"Sample preparation failed"** section of this handbook.

OR

“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

a. Check the entire workflow and test set-up to look for any user errors, then repeat the test
    i. e.g. have the right color pipettes and solutions been used with the correct tubes?
b. Ensure the positive and negative controls are inserted into the correct wells of your q16.
c. 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 DNA. If the Positive Control works, the mock sample will now be called as a negative result.

OR

“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

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

Primerdesign™ Ltd

OnDemand Easy Kit

RNA Kit

50 reaction
for use on the genesig® q16

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Kit Contents

N.B. *Once resuspended the Kits must remain at* ***-20ºC*** *until ready to use.*

Target specific primer/probe mix (BROWN)

Lyophilised oasig™ OneStep Master Mix

Lyophilised oasig™ Master Mix resuspension buffer (BLUE lid)

Target 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

OnDemand Easy Kit (RNA Kit): at a glance guide

For each Sample RNA test

Component	Volume	Lab-in-a-box pipette	
Target Primer/Probe mix	5 µl		
Your RNA sample	5 µl		
oasig OneStep Master Mix	10 µl		

For each positive control

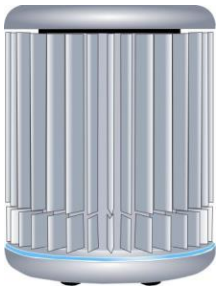
Component	Volume	Lab-in-a-box pipette	
Target Primer/Probe mix	5 µl		
Positive control template	5 µl		
oasig OneStep Master Mix	10 µl		

For each negative control

Component	Volume	Lab-in-a-box pipette	
Target Primer/Probe mix	5 µl		
Water	5 µl		
oasig OneStep Master Mix	10 µl		

Reagents and equipment to be supplied by the user

genesig® q16 instrument



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genesig® Easy DNA/RNA Extraction Kit

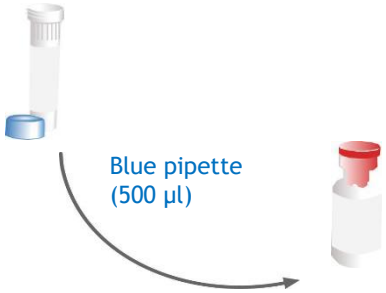
This Kit is designed to work well with all processes that yield high quality RNA but the genesig Easy extraction method is recommended for ease of use.

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.

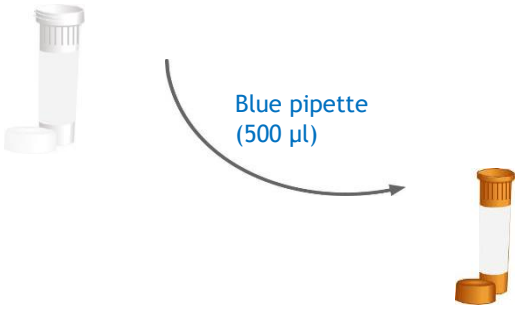
Step-by-step guide

1. Resuspend the test components



a. Use the blue pipette to transfer 500 µl* of the oasig Master Mix resuspension buffer (blue lid) into the tube of Lyophilised oasig OneStep Master Mix
b. Mix well by gently swirling (lid on)

*Transferring **525 µl** 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.

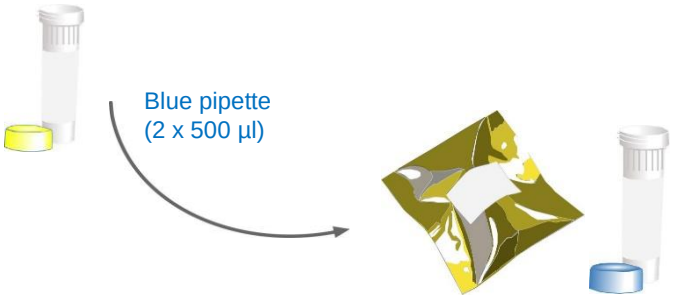


c. Use the blue pipette to transfer 500 µl of water (white lid) into the brown tube labelled target primers/probe.
d. Cap and shake tube to mix. A thorough shake is essential to ensure that all components are resuspended.
Failure to mix well can produce poor Kit performance.

These Failure to mix well can produce poor Kit performance.

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



a. Use the blue pipette to transfer 1000 µl (2 x 500 µl) of template preparation buffer (yellow Lid) into the Internal Extraction Control RNA tube (blue Lid)

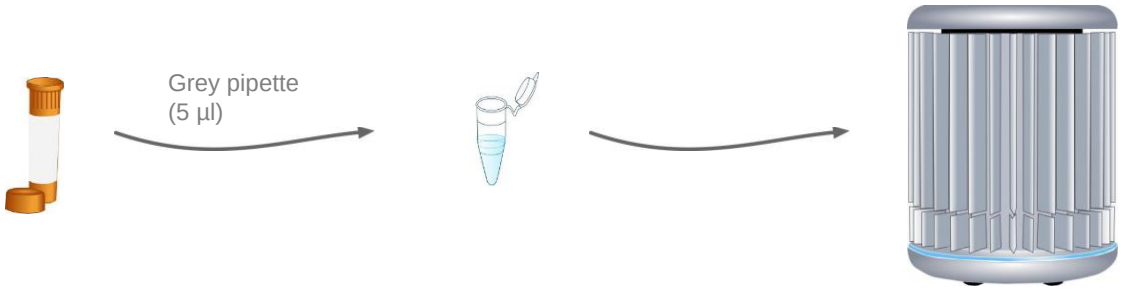
b. Cap and shake tube to mix.

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

OR

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

3. Add primer/probe mix to all reaction tubes

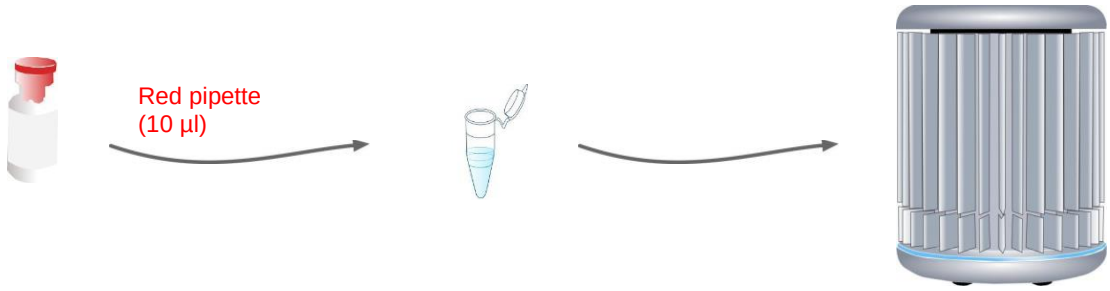


a. For every reaction to be run, use the grey pipette to add 5 µl of your Target primers/probe mix (Brown Lid) to every tube.

#PRIMERDESIGNTOPTIP

- 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

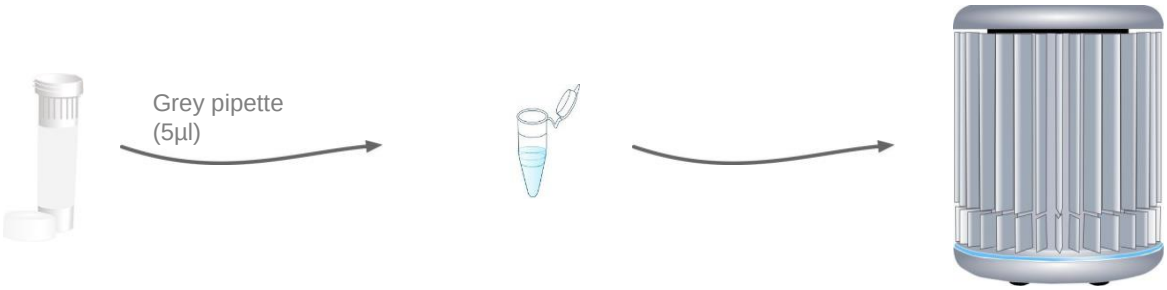


a. For every reaction to be run, use the red pipette to add 10 µl of the oasig OneStep Master Mix to the tubes containing primer/probe mix.

#PRIMERDESIGNTOPTIP

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

5. Negative control



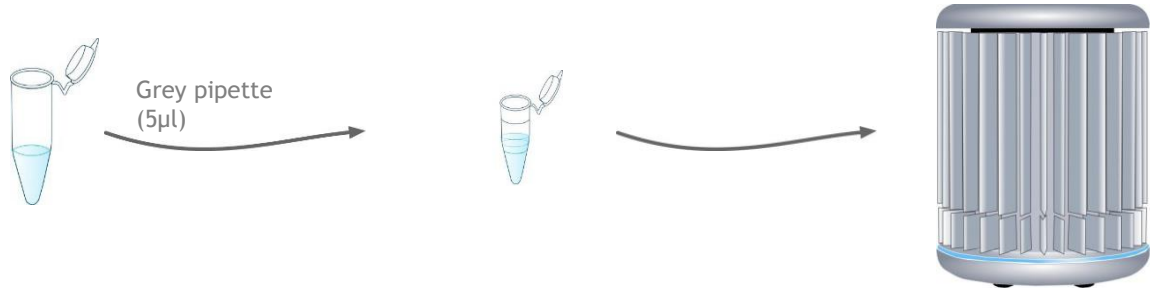
For each test you will require a negative control. Instead of RNA, water is used. This sample should typically prove negative thus proving that all of your positive samples really are positive.

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

#PRIMERDESIGNTOPTIP

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

6. Set up a test



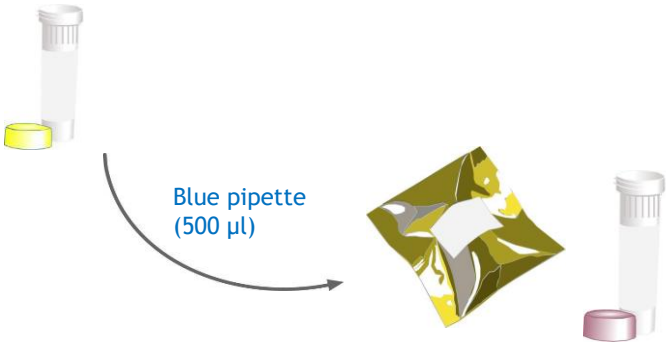
a. For each sample you wish to analyse, use the grey pipette to add 5 µl of your sample to the required reaction tubes.

b. Close these tubes after adding the sample. Always change pipette tips between samples.

#PRIMERDESIGNTOPTIP

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

7. Positive control



a. Use the blue pipette to transfer 500 µl of template preparation buffer into the positive control template tube.

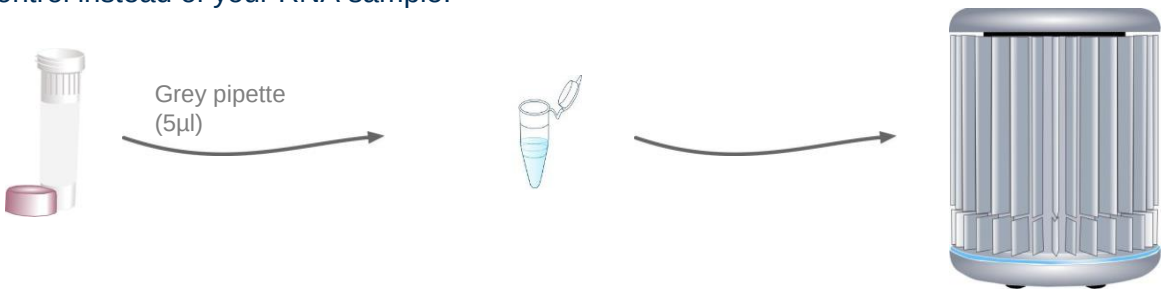
b. Cap and shake tube to mix.

#PRIMERDESIGNTOPTIP

- Each time you run a test you will require a positive control. 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

a. To create a positive control reaction simply use the grey pipette to transfer 5 µl of the positive control instead of your RNA sample.

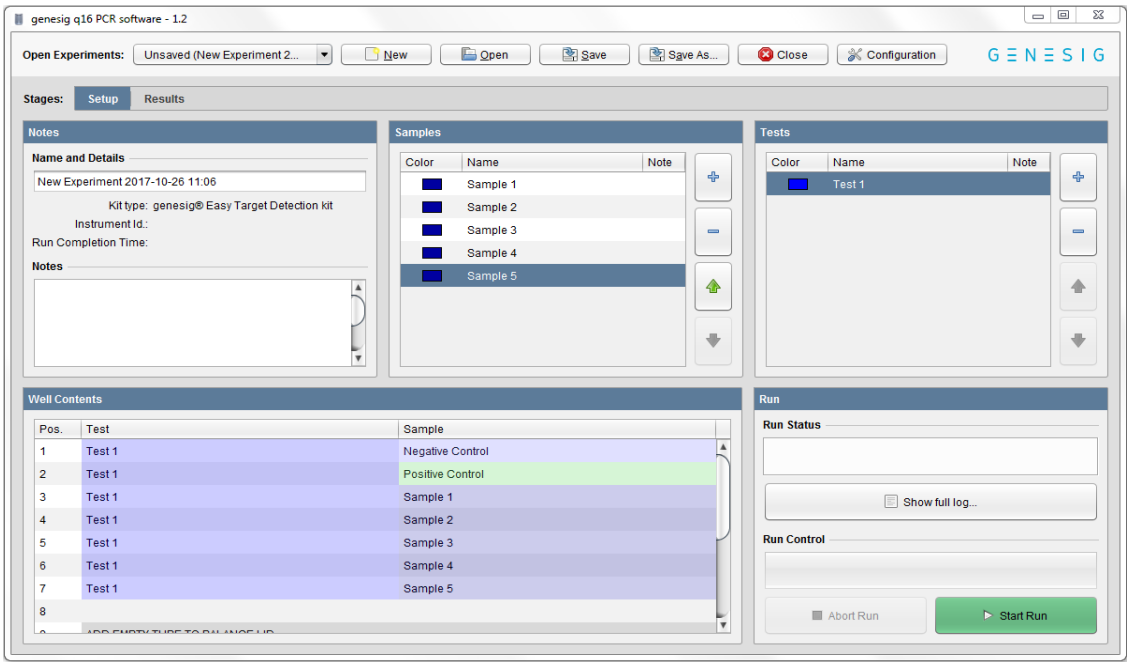


#PRIMERDESIGNTOPTIP

- 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.
- 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.



#PRIMERDESIGNTOPTIP

- 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

9. What do my results mean?

Analysis of your data is carried out automatically by the genesig q16.

a. 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.

OR

"Negative"

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

OR

"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 / RNA 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

a. 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
b. 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.

c. To 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 DNA. The result for the Negative Control and the mock sample should be negative indicating that contamination is no longer present.
d. 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
    i. The best workflow involves setting up all the test components (excluding the positive control template) in the clean area
    ii. 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.
    iii. For extra security the Negative Control can be completely prepared and sealed in the clean area.
    iv. All work areas should be decontaminated regularly with DNA remover.

OR

"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

a. Check the sample preparation protocol for any user errors then repeat. 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.
b. 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".
c. 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.

OR

"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

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

OR

“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

a. Check the entire workflow and test set-up to look for any user errors, then repeat the test
    i. e.g. have the right color pipettes and solutions been used with the correct tubes?
b. Ensure the positive and negative controls are inserted into the correct wells of your q16.
c. 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.

OR

“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

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

Primerdesign™ Ltd

OnDemand Kit

Speciation Kit

50 reaction

Easy Kit

for use on the genesig® q16

For Research Use Only. Not for use in diagnostic procedures

Kit Contents

***N.B.** Once resuspended the Kits must remain at **-20ºC** until ready to use*

Target specific species-specific primer/probe mix (BROWN)

Lyophilised oasig™ Master Mix

Lyophilised oasig™ Master Mix resuspension buffer (BLUE lid)

Target positive control template (RED lid)

RNase/DNase free water (White Lid)

Template preparation buffer (YELLOW lid)

54 x genesig® q16 reaction tubes

OnDemand Easy Kit (Speciation Kit): at a glance guide

For each Sample test

Component	Volume	Lab-in-a-box pipette	
Species reaction mix	10 µl		
Your DNA sample	10 µl		

For each positive control

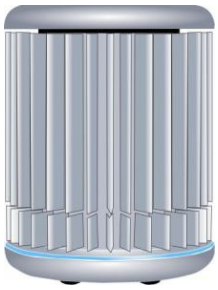
Component	Volume	Lab-in-a-box pipette	
Species reaction mix	10 µl		
Positive control template	10 µl		

For each negative control

Component	Volume	Lab-in-a-box pipette	
Species reaction mix	10 µl		
Water	10 µl		

Reagents and equipment to be supplied by the user

genesig® q16 instrument



The genesig q16 is a revolutionary instrument launched by Primerdesign Ltd. The instrument is designed to accompany the genesig® Easy product range which includes Kits for more than 600 different DNA testing applications. The q16 is designed to make DNA testing affordable and easy for anyone in any business.

genesig® Easy DNA/RNA Extraction Kit

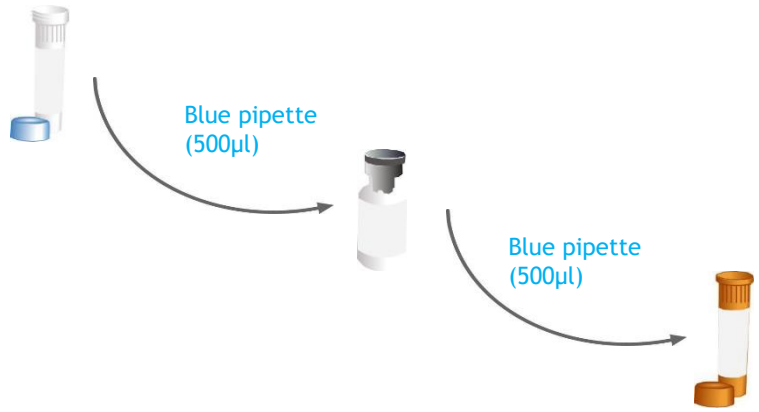
This Kit is designed to work well with all processes that yield high quality DNA but the genesig Easy extraction method is recommended for ease of use.

genesig® Lab-In-A-Box

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

Step-by-step guide

1. Create your reaction mix



Use the blue pipette to

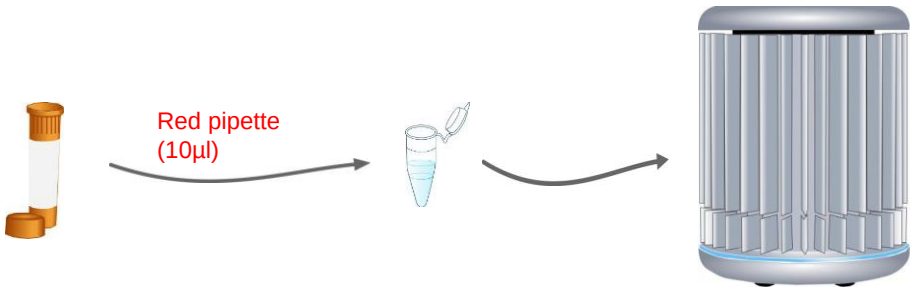
a. Transfer 500 µl* of the oasig Master Mix resuspension buffer (blue lid) into the tube of Lyophilised oasig Master Mix
b. Mix well by gently swirling (lid on)
c. Then transfer all that master mix into the brown tube labelled Target primers/probe (Brown Lid)
d. Thorough shake is essential to ensure all components are resuspended
e. Leave to stand for **5 minutes**. Now your reaction mix is ready to use
f. Store the reaction mix in the freezer (-20°C) from hereon

Failure to mix well can produce poor Kit performance.

Once resuspended do not expose genesig Easy Kit to temperatures above -20°C for longer than 30 minutes at a time.

*Transferring 525µl of the oasig Master Mix resuspension buffer to your oasig 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.

2. Add reaction mix to all reaction tubes

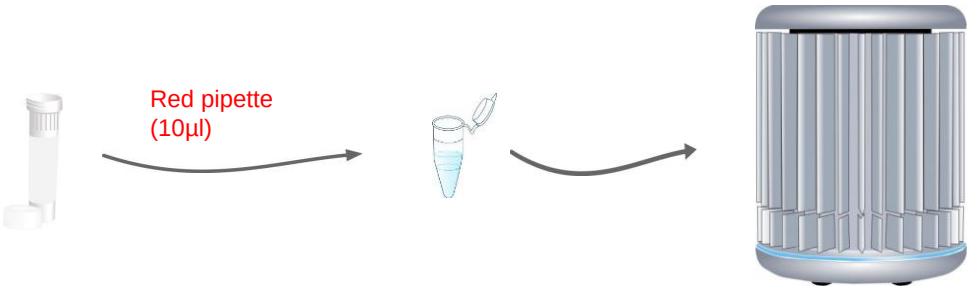


a. For every reaction to be run, use the red pipette to add 10 µl of your Species-specific reaction mix to every tube.

#PRIMERDESIGNTOPTIP

- Always pipette the reaction mix directly into the bottom of the tube.
- You can label the tube lids to aid your reaction setup but avoid labelling tube sides.

3. Negative control



a. To create a negative control reaction simply use the red pipette to add 10 µl of the water to the required reaction tube
b. Close these tubes after adding the water

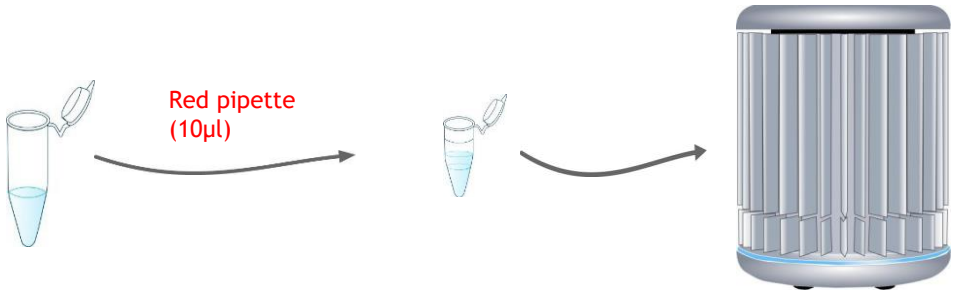
For each test you will require a negative control. Instead of DNA, water is used. This sample should typically prove negative thus proving that all your positive samples really are positive.

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.

#PRIMERDESIGNTOPTIP

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

4. Set up a test

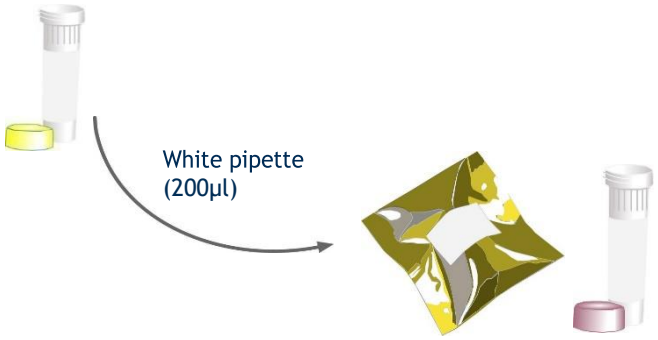


a. For each sample you wish to analyse, use the red pipette to add 10 µl of your DNA sample to the required reaction tubes *(**N.B.** Always change pipette tips between samples)*
b. Close these tubes after adding the sample.

#PRIMERDESIGNTOPTIP

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

5. Positive control



a. Use the white pipette to transfer 200 µl of template preparation buffer into the positive control template tube.

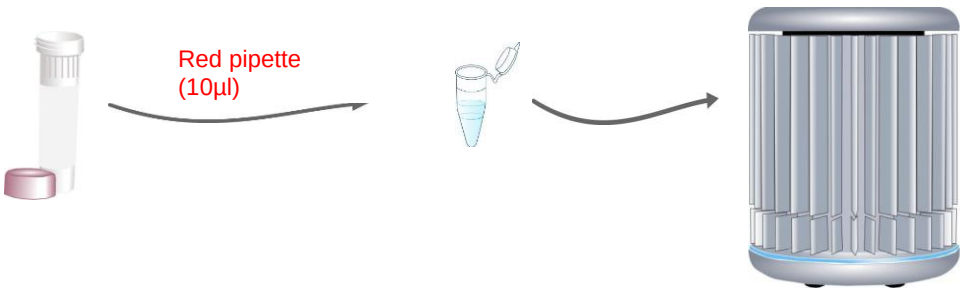
b. Cap and shake tube to mix.

Why run a Positive Control?

Each time you run a test you will require a positive control. This is a small portion of DNA from your species 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 DNA is present in the positive control. It uses this information to internally calibrate every test. This is essential to give accurate information about the percentage of DNA in your sample that is from your species of interest.

c. To create a positive control reaction, simply use Red Pipette 10 µl of the positive control instead of your DNA sample.

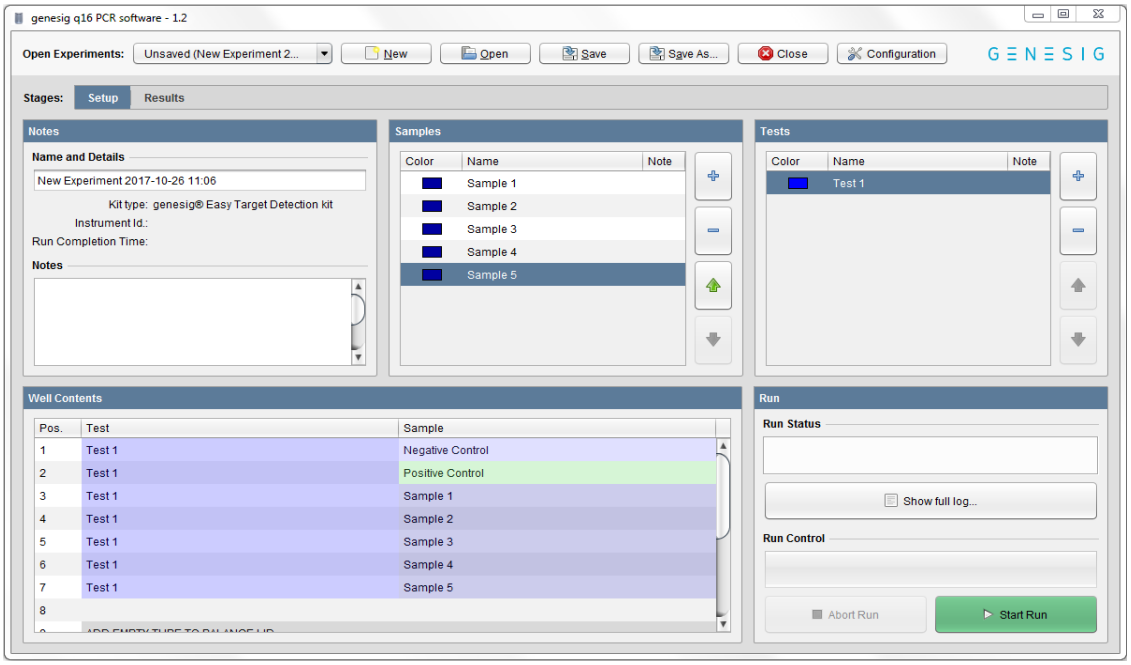


#PRIMERDESIGNTOPTIP

- 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.

6. 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.



#PRIMERDESIGNTOPTIP

- 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

7. What do my results mean?

Analysis of your data is carried out automatically by the genesig q16.

a. 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 percentage.

OR

"Negative"

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

OR

"Test contaminated"

Explanation
The Negative Control should be completely free of any DNA. If you see this error message it means that at some point during the setup, the Negative Control has been contaminated with DNA 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. 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.

a. 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
b. 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.
c. To 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 DNA. The result for the Negative Control and the mock sample should be negative indicating that contamination is no longer present.
d. An ideal lab set-up has a 'Clean area' where the test reagents are prepared and a 'sample area' where DNA samples and the Positive Control template are handled
    i. The best workflow involves setting up all the test components (excluding the

positive control template) in the clean area

ii. 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.

iii. For extra security the Negative Control can be completely prepared and sealed in the clean area.

iv. All work areas should be decontaminated regularly with DNA remover.

OR

"Test failed"

Explanation - If positive control has failed

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

a. Check the entire workflow and test set-up to look for any user errors, then repeat the test
    i. e.g. have the right color pipettes and solutions been used with the correct tubes?

b. Ensure the positive and negative controls are inserted into the correct wells of your q16.

c. 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 DNA. If the Positive Control works, the mock sample will now be called as a negative result.

Explanation - If positive control has passed

The test has failed because the sample is not suitable for qPCR testing. This particular sample has failed because it contains one or more factors that are inhibitory to PCR. This has compromised accuracy and precision of the quantitative reporting, resulting in a reported speciation percentage greater than 100%.

Solutions

a. Dilute the extracted sample 1:10 in water to "dilute out" the PCR inhibitors.

b. Check the sample preparation protocol to look for any user errors, then repeat.

c. PCR inhibitors can result from overloading the DNA/RNA sample preparation protocol with too much starting material. Try reducing the amount of starting material (by a factor of 2) then repeat.

OR

"Positive result lower than test sensitivity"

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, if the calculated percentage falls outside the accurate range for the test the exact percentage cannot accurately be calculated. Nonetheless a semi-quantitative result can be reported based on the "% sensitivity" column. e.g. If the "% sensitivity" is reported at "1%" then the sample can be reported as "positive test with less than 1% presence of

species of interest". If you require more accurate information for your sample, then proceed with the solutions below.

Solutions

a. A higher quality of sample containing more DNA will yield a more sensitive test. Try increasing the concentration of sample that is added to the Sample Prep Solutions stage during the extraction.
b. If you cannot increase the amount of sample, check the sample preparation protocol to look for any user errors then repeat.
c. 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.

OR

"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

a. For appropriate solutions, read both the "Test failed" and "Test contaminated" sections of this handbook.

"Low level of universal target DNA"

Explanation

The test has failed because either the sample quality or quantity was too low. This has been identified by the universal target and may be caused by the sample having been compromised or a low amount of the universal target being present in the starting material. In either case the sample will not be fit for analysis.

Solutions

a. Try increasing the amount of sample that is added to the Sample Prep Solution stage during the extraction.
b. If you cannot increase the amount of sample, check the sample preparation protocol to look for any user errors then repeat.
c. 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.

OR

“Positive - Caution, low level of universal target DNA”

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 quantification has failed because either the sample quality or quantity was too low. The signal produced by the universal target acts as an endogenous control, identifying the quantity of DNA in the sample form which the species percentage is calculated. In this case the total level of DNA was not high enough. This will occur if the sample quality has been compromised or if a low amount of universal target was present in the starting material.

Solutions

For appropriate solutions read the “Low level of universal target DNA” section of this handbook