Primerdesign™ Ltd

Aggregatibacter actinomycetemcomitans

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 DNA test

Component	Volume	Lab-in-a-box pipette	
A.actino reaction mix	10 µl	●	
Your DNA sample	10 µl	●	

For each positive control

Component	Volume	Lab-in-a-box pipette	
A.actino reaction mix	10 µl	●	
Positive control template	10 µl	●	

For each negative control

Component	Volume	Lab-in-a-box pipette	
A.actino reaction mix	10 µl	●	
Water	10 µl	●	

Kit Contents

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

- **Lyophilised oasig™ Master Mix**

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

- **A.actino positive control template (RED lid)**

- **Internal extraction control (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

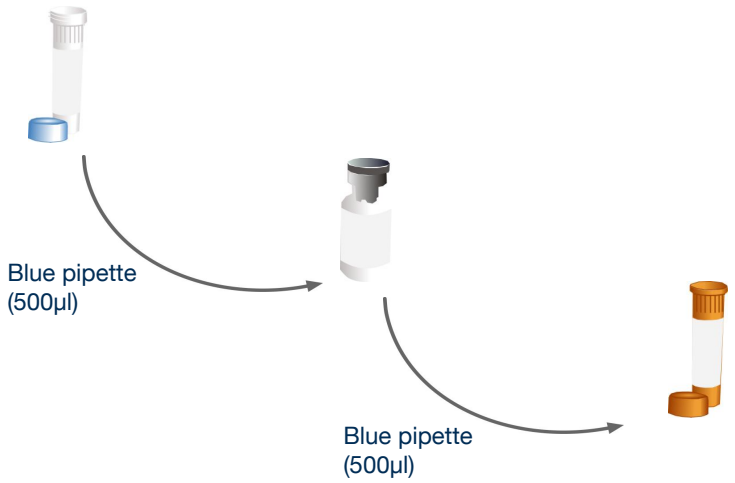
This kit is designed to work well with all processes that yield high quality RNA and DNA but the genesig Easy extraction method is recommended for ease of use.

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. Create your reaction mix



Use the blue pipette to transfer 500µl* of the oasig Master Mix resuspension buffer into the tube of lyophilised oasig Master Mix and mix well by gently swirling. Then transfer all of that master mix into the brown tube labelled A.actino primers/probe.

*Transfering 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.

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.**

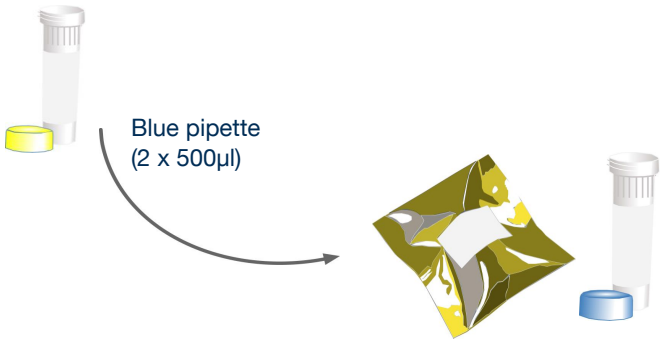
Leave to stand for 5 minutes. Now your reaction mix is ready to use.

Store the reaction mix in the freezer from hereon.

Top tip

- Ensure that the reaction 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



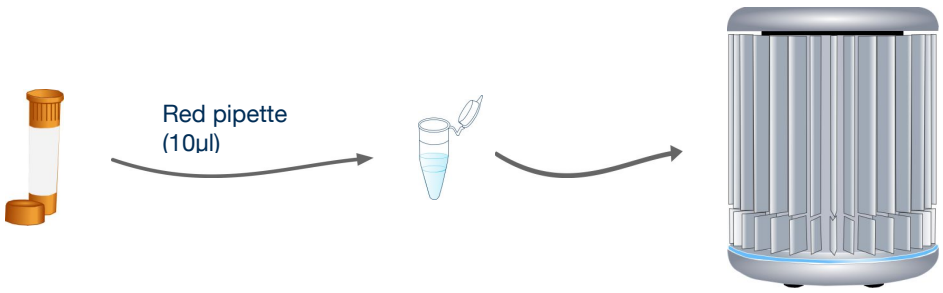
Use the blue pipette to transfer 1000µl (2 x 500µl) of template preparation buffer into the Internal Extraction Control DNA tube. Cap and shake tube to mix.

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

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

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

3. Add reaction mix to all reaction tubes

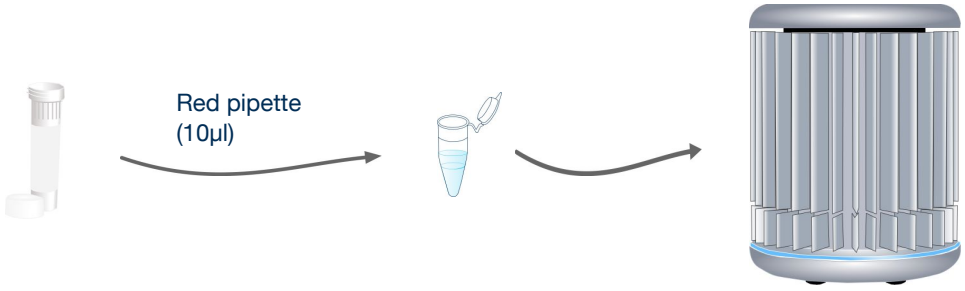


For every reaction to be run, use the red pipette to add 10µl of your A.actino reaction mix to every tube.

Top tip

- 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.

4. Negative control



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

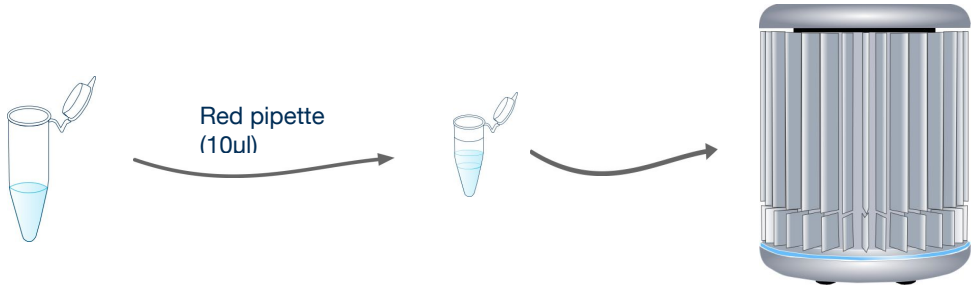
To create a negative control reaction simply use the red pipette to add 10µl of the water to the required reaction tubes. Close these tubes after adding the water.

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.

5. Set up a test

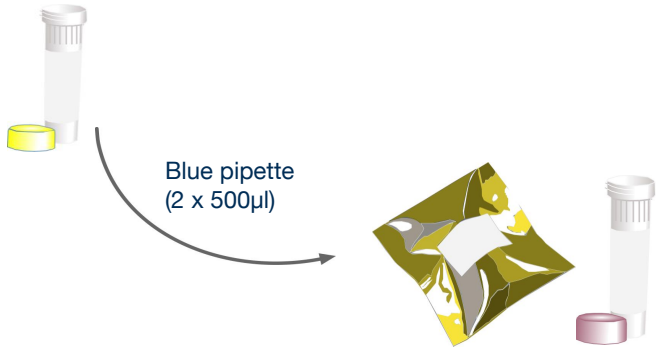


For each sample you wish to analyse, use the red pipette to add 10µl of your DNA sample to the required reaction tubes. Close these tubes after adding the sample. Always change pipette tips between samples.

Top tip

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

6. Positive control

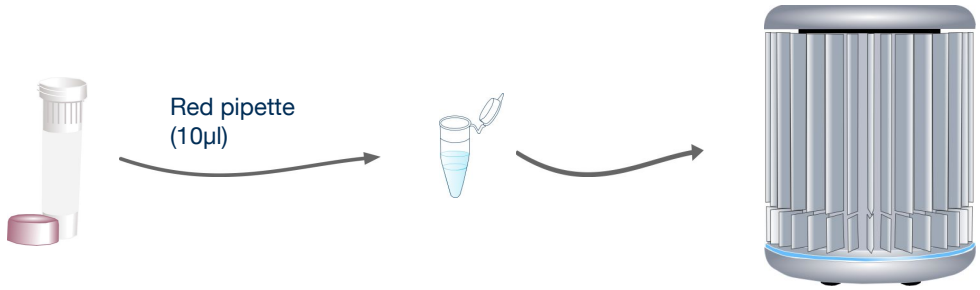


Use the blue pipette to transfer 1000µl (2 x 500µl) of template preparation buffer into the positive control template tube. Cap and shake tube to mix.

Each time you run a test you will require a positive control. This is a small portion of 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 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 in your sample.

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



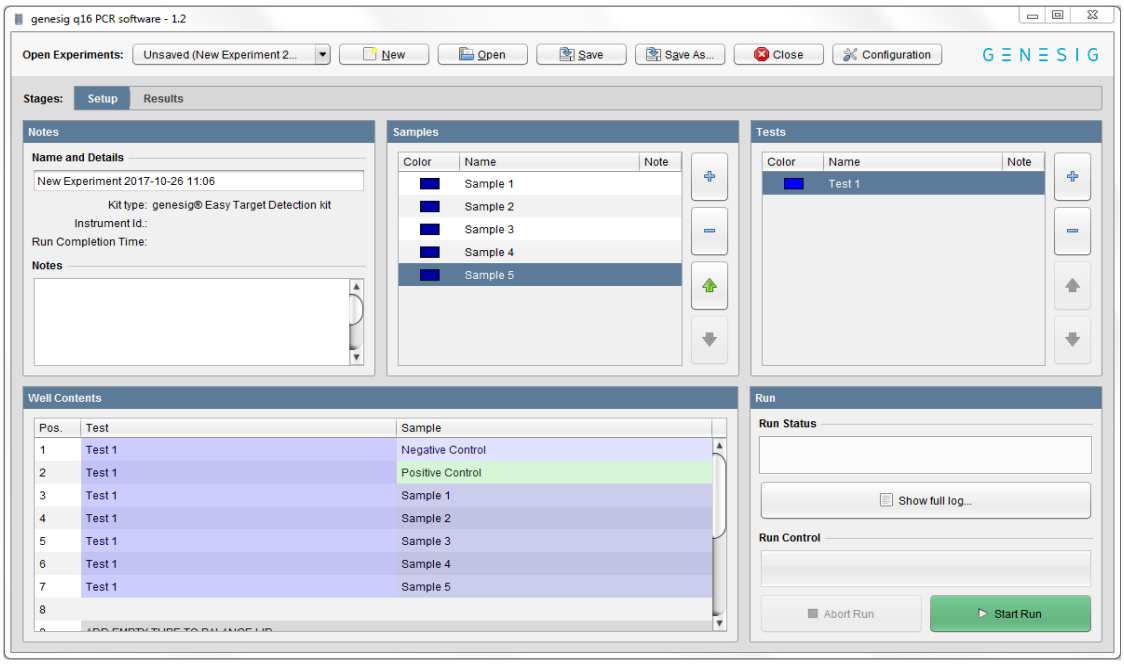
Take great care when setting up your positive control. The positive control template has the potential to give you a false positive signal in your other samples. Set positive controls up last after all other sample tubes are closed. Always change pipette tips between samples. You may even choose to set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

Top tip

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

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.



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. 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

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 DNA. 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 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 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.

“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.

“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 DNA. 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

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

Aggregatibacter actinomycetemcomitans

Aggregatibacter actinomycetemcomitans is a non-motile, rod-shaped, Gram-negative bacterium. This bacterium is also a facultative anaerobe which can grow under aerobic or anaerobic conditions. The 43Kbp genome is arranged in a circular configuration and contains up to 60 open reading frames. The outer surface of the cell includes appendages known as fimbriae which are used for host cell adhesion.

A. actinomycetemcomitans inhabits the human oral cavity where it adheres to the mucosal tissue of the gum using fimbriae. Here, they are able to create a biofilm with other resident bacterial species that if not treated can cause periodontitis or gingivitis.
The bacterium produces leukotoxin which can be found on the outer bacterial membrane and is used to avoid the host cell immune response. This evasion allows the bacteria to stimulate a chronic inflammatory response that leads to damage of the periodontium which includes connective tissue, ligaments and bone.

The damage to the periodontium that is caused by A. actinomycetemcomitans results in redness, bleeding or swelling of the gums which progresses to recession of the gums that can lead to deep pockets forming between the teeth when the attached gingiva is destroyed. Eventually the teeth will become lose and fall out.

Specificity

The Primerdesign genesig Kit for Aggregatibacter actinomycetemcomitans (A.actino) genomes is designed for the in vitro quantification of A.actino 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.

The haemoglobin binding protein gene has previously been identified as a highly specific market for A. actinomycetemcomitans (J. Periodont Res 2008; 43: 352-359). The primers and probe have 100% homology with over 95% of NCBI database sequences.

EF142372.1, EF142371.1, EF142368.1, EF142367.1, EF142366.1, EF142364.1, EF142363.1, AF359451.1, AF359444.1, AF359442.1, EF142443.1, EF142442.1, EF142441.1, EF142440.1, EF142439.1, EF142438.1, EF142437.1, EF142436.1, EF142435.1, EF142434.1, EF142433.1, EF142432.1, EF142431.1, EF142430.1, EF142429.1, EF142428.1, EF142427.1, EF142426.1, EF142425.1, EF142424.1, EF142423.1, EF142422.1, EF142421.1, EF142420.1, EF142419.1, EF142418.1, EF142417.1, EF142416.1, EF142415.1, EF142414.1, EF142413.1, EF142412.1, EF142411.1, EF142410.1, EF142409.1, EF142408.1, EF142407.1, EF142406.1, EF142405.1, EF142404.1, EF142403.1, EF142402.1, EF142401.1, EF142400.1, EF142399.1, EF142398.1, EF142397.1, EF142396.1, EF142395.1, EF142394.1, EF142393.1, EF142392.1, EF142391.1, EF142390.1, EF142389.1, EF142388.1, EF142387.1, EF142386.1, EF142385.1, EF142384.1, EF142383.1, EF142382.1, EF142381.1, EF142380.1, EF142379.1, EF142378.1, EF142377.1, EF142376.1, EF142375.1, EF142374.1, EF142373.1, EF142370.1, EF142369.1, EF142365.1, AF359450.1, AF359449.1, AF359448.1, AF359447.1, AF359446.1, AF359445.1, AF359443.1

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 A.actino 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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