Primerdesign

Z-Path-T.pallidum-EASY

Treponema pallidum

Kit version: 1

Target region:
polA gene

genesig® Easy DNA Kit

for use on the genesig® q16
50 reactions

GENESIG
Kits by Primerdesign

For general laboratory and research use only

Product Description

This genesig® Easy qPCR detection kit targets the polA gene from Treponema pallidum (T. pallidum). T. pallidum is a gram-negative bacterium of the Spirochaetes phylum. The bacteria are approximately 6-20µm in length with a helical shape. Endoflagella found in the periplasmic space between the outer and inner membrane enable corkscrew motility. The bacterial genome is one double-stranded DNA circular chromosome of around 1Mbp in length that codes for over 1000 proteins. This bacterium is the parasite responsible for causing Syphilis and is also thought to increase the likelihood of transmission of HIV.

T. pallidum acquires all of its energy through glycolysis, but relies on a host for nutrients and therefore cannot survive in the absence of the host cells. The initial site of infection is the epithelial cells of the genitals, although the bacterium can spread throughout the body to any tissue or organ. The infection is a sexually transmitted disease and is spread by contact with skin lesions or exposed mucus membranes. However, congenital syphilis is transmitted from mother to fetus. Previous infections with T. pallidum offer no immunity, but the infection can be treated with antibiotics such as penicillin which it has not shown resistance to.

T. pallidum infection progresses through 4 stages if untreated: Primary; secondary; latent and tertiary. Primary infection presents in a chancre lesion at the site where the bacteria penetrate the epithelial cell. These indurated ulcers are clean and painless and can last up to 5 weeks. If left untreated the second stage of the infection follows with a rash appearing approximately 6 weeks after the initial infection, accompanied by skin lesions. People infected may also experience tiredness, fever, headaches, sore throat, loss of appetite and swollen glands which can last up to 6 weeks. Several months later symptoms disappear although the bacterium is still present, this is the latent phase where transmission rates decrease significantly. If the infection is not treated, the tertiary phase may begin up to several years later. At this stage, syphilis can affect the bones, the cardiovascular system and the central nervous system causing disease.

Specificity

The kit is designed for the in vitro quantification of T. pallidum genomes and to have a broad detection profile.

The polA gene has previously been established in the scientific literature as a reliable marker for the specific detection of T. pallidum (D.Leslie et.al. 2007). The primers and probe have 100% homology with over 95% of the reference sequences for polA from T. pallidum currently in NCBI database (including CP000805.1 AE000520.1 U57757.1).

The dynamics of genetic variation means that new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit, please send an email to techsupport@primerdesign.co.uk and our team will answer your question.

genesig® Easy: at a glance guide

For each DNA test

Component	Volume	Lab-in-a-box pipette	
T.pallidum reaction mix	10 µl		
Your DNA sample	10 µl		

For each positive control

Component	Volume	Lab-in-a-box pipette	
T.pallidum reaction mix	10 µl		
Positive control template	10 µl		

For each negative control

Component	Volume	Lab-in-a-box pipette	
T.pallidum reaction mix	10 µl		
Water	10 µl		

Kit Contents

Quantity	Component	Tube	Cap Colour
1	**T.pallidum primer/probe mix (including internal extraction control primers and probes) (50 reactions)** FAM and VIC labelled as standard		**BROWN**
1	**T.pallidum positive control template**		**RED** (in foil wrapper)
1	**oasig® Lyophilised 2X qPCR Master Mix**		**SILVER**
1	**oasig® resuspension buffer** for resuspension of the Master Mix		**BLUE**
1	**Internal extraction control DNA template**		**BLUE** (in foil wrapper)
1	**RNase/DNase free water** for negative control		**WHITE**
2	**Template preparation buffer** for resuspension of internal control template and positive control template		**YELLOW**
54	**genesig® q16 reaction tubes**		**N/A**

Reagents and equipment to be supplied by the user

genesig® q16 instrument

genesig® Easy Extraction Kit

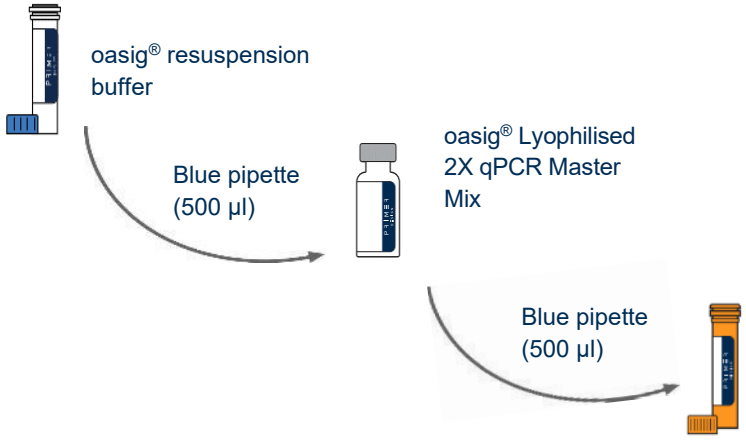
This kit is designed to work well with all processes that yield high-quality nucleic acid but the genesig® Easy extraction method is recommended for ease of use.

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 transfer 500 µl* of the oasig® 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 T.pallidum primers/probe.

*Transferring 525 µl of the oasig® resuspension buffer to your oasig® Lyophilised 2X qPCR 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.

Cap and shake 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 10 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 and pipetting up and down 10 times.
- Once resuspended do not expose the genesig® Easy kit to temperatures above -20°C for longer than 30 minutes at a time.

2. Internal extraction control template



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

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

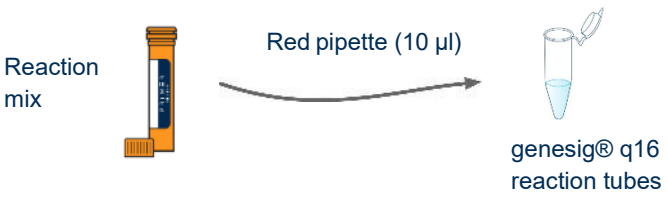
If you are using an extraction kit:

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

3. Add reaction mix to all reaction tubes

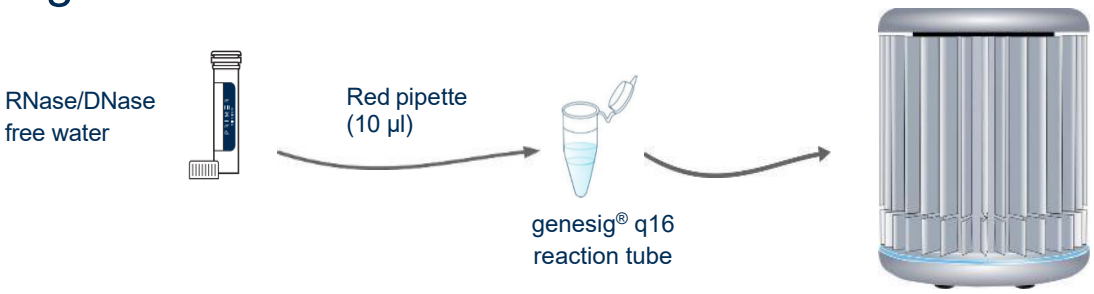


For every reaction to be run, use the red pipette to add 10 µl of your T.pallidum reaction mix to every genesig® q16 reaction tube.

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 DNA, water is used. The negative control sample should give a negative test result and thereby prove that any positive samples really are positive, and not tested positive due to contamination.

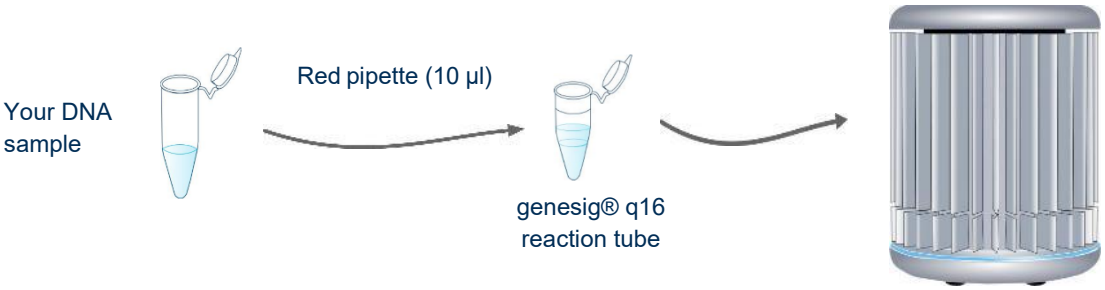
To create a negative control reaction simply use the red pipette to add 10 µl of the water to the required reaction tube (already containing primer/probe mix and oasig® Lyophilised 2X qPCR Master Mix). Close this tube after adding the water.

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

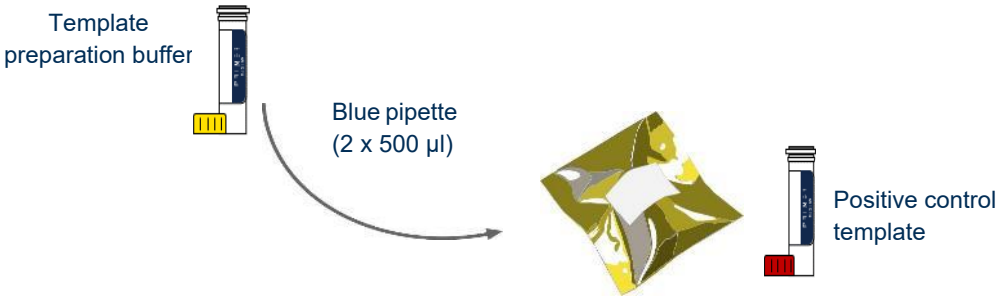


For each sample you wish to analyse, use the red pipette to add 10 µl of your DNA sample to the required reaction tube (already containing primer/probe mix and oasig® Lyophilised 2X qPCR Master Mix). Close these tubes after adding the sample. Always change pipette tips between samples.

Top tip

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

6. Positive control

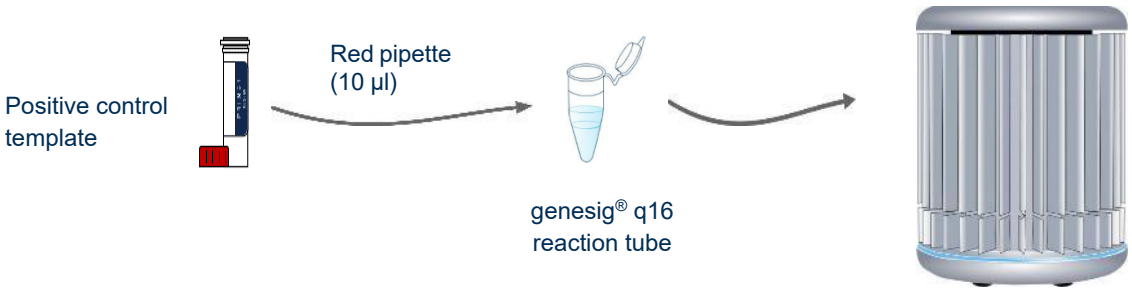


Use the blue pipette to transfer 1000 µl (2 x 500 µl) of template preparation buffer into the positive control template tube. Cap and shake tube to mix.

Each time you run a test you will require a positive control. This is a small portion of DNA 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 many copies of the target are present in the positive control. Therefore, it can automatically compare your sample of interest with the positive control to calculate the amount of target DNA in your sample.

To create a positive control reaction simply use 10 µl of the positive control template instead of your DNA sample and add this to the required reaction tube (already containing primer/probe mix and oasig® Lyophilised 2X qPCR Master Mix). Close this tube after adding the positive control template.



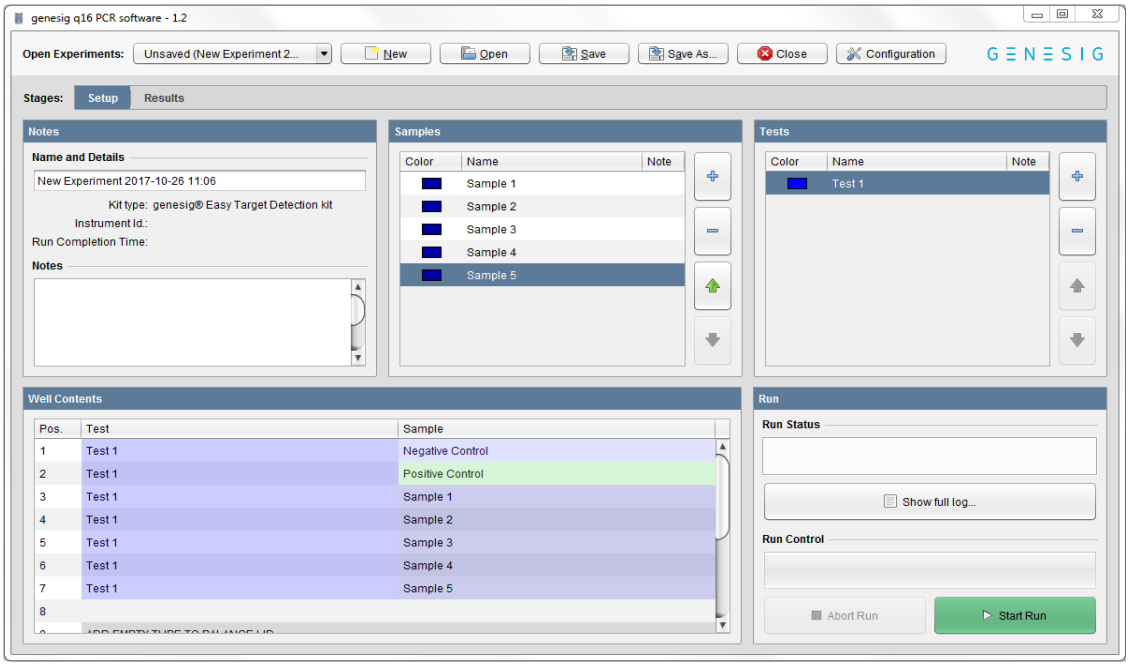
Take great care when setting up your positive control. The positive control template has the potential to give you a false positive signal in your other samples. Set positive controls up last after all other sample tubes are closed. Always change pipette tips between samples. You may even choose to set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

Select the genesig Easy Target Detection Kit module within the software. Place the reaction 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.

What do my results mean?

Analysis of your data is carried out automatically by the genesig® q16 software. 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 internal control template 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 template 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 therefore not 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 mentioned above under "Sample preparation failed".

“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.

Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances. Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA integrity.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired.

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