



Primerdesign™ Ltd

Enterocytozoon bienersi

genesig® Easy Kit
for use on the genesig q16

50 reaction


DNA testing

Everything...
Everyone...
Everywhere...


For general laboratory and research use only

genesig[®] Easy: at a glance guide


For each DNA test

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

For each positive control

Component	Volume	Lab-in-a-box pipette	
E. bienewsi reaction mix	10 µl	●	
<u>Positive control template</u>	10 µl	●	

For each negative control

Component	Volume	Lab-in-a-box pipette	
E. bienewsi reaction mix	10 µl	●	
<u>Water</u>	10 µl	●	

Kit Contents



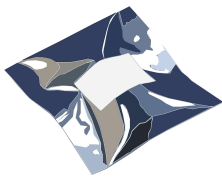
- **E. bienensei specific primer/probe mix (BROWN)**



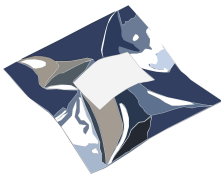
- **Lyophilised oasig MasterMix**



- **Lyophilised oasig MasterMix resuspension buffer (BLUE lid)**



- **E. bienensei positive control template (RED lid)**



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



- **RNAse/DNAse free water (WHITE lid)**



- **50 x genesig q16 reaction tubes**

Reagents and equipment to be supplied by the user

genesig® q16 instrument

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 of the pipettes, tips, 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.

Introduction to Enterocytozoon bienewsi

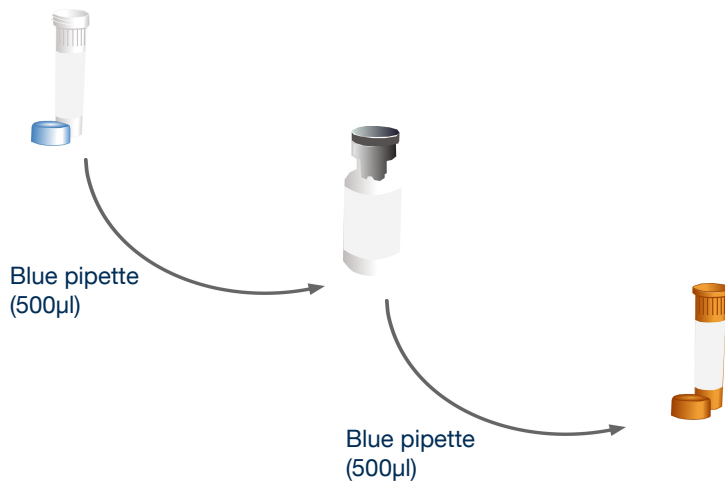
Enterocytozoon bienewsi, a fungus of the microsporidia phylum is an obligate intracellular parasite that infects intestinal epithelial cells. The linear DNA genome is estimated at 40 Mb, organized in 3 or 4 chromosomes with a total of 3,804 genes, 1,702 of these encoding functional proteins. This species infects human and other mammals causing microsporidiosis and many associated opportunistic infections which have had a serious economic impact on the swine industry particularly.

Infective E. bienewsi spores are hardy and can survive in the environment for long periods of time. The oval shape is created by two extracellular walls surrounding a membrane containing the sporoplasm, the infectious material. Upon ingestion with contaminated food stuffs or water, the extrusion apparatus comprising an anchoring disk, polar tubule, and polaroplast extend from the spore piercing the host cell membrane. The sporoplasm is then injected through this apparatus into the host cell, primarily the intestinal epithelium. Within the host cell, proliferation by sporogony occurs in the cytoplasm and is followed by maturation. The resultant new spores cause host cell death by disrupting the host cell membrane leading to release into the intestine allowing for subsequent infection of surrounding cells or excretion and transmission.

E. bienewsi causes microsporidiosis in humans, specifically in immune-compromised individuals and has been linked with the wasting and chronic diarrhoea seen in individuals with AIDS. Other symptoms include urinary tract infections and bowel perforation, cough and laboured breathing due to infection of the lungs, and inflammation of the brain and muscle tissue in cases with the fungi has spread to other parts of the body. Fumagillin and Albendazole have been effective in treatment to a certain extent along with treatments utilising inhibitors of chitin synthase enzymes.

Step-by-step guide

1. Create your reaction mix

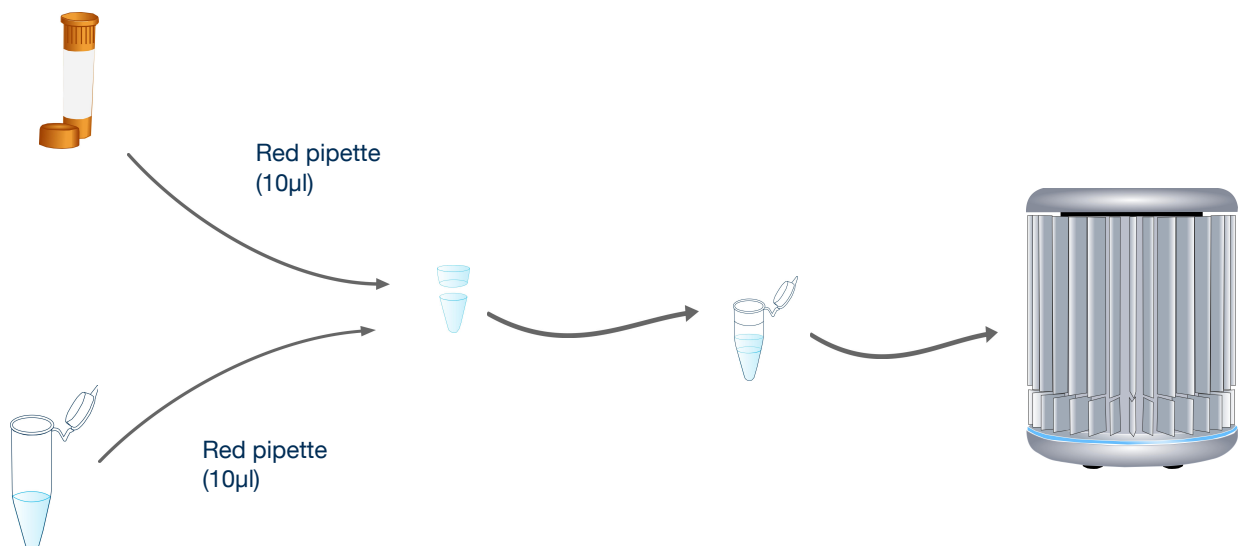


Use the blue pipette to transfer 500µl of the oasig mastermix resuspension buffer in to the tube of lyophilised oasig mastermix. Then transfer all of that mastermix in to the brown tube labelled *E. bienersi* primers/probe.

Now your reaction mix is ready to use.

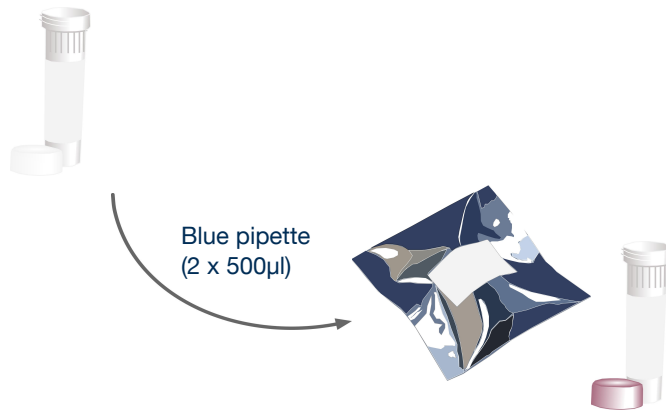
Store the reaction mix in the freezer from hereon.

2. Set up your test



For each sample you wish to analyse, use the red pipette to combine 10µl of your reaction mix with 10µl of your DNA sample in the reaction tubes provided.

3. Positive control

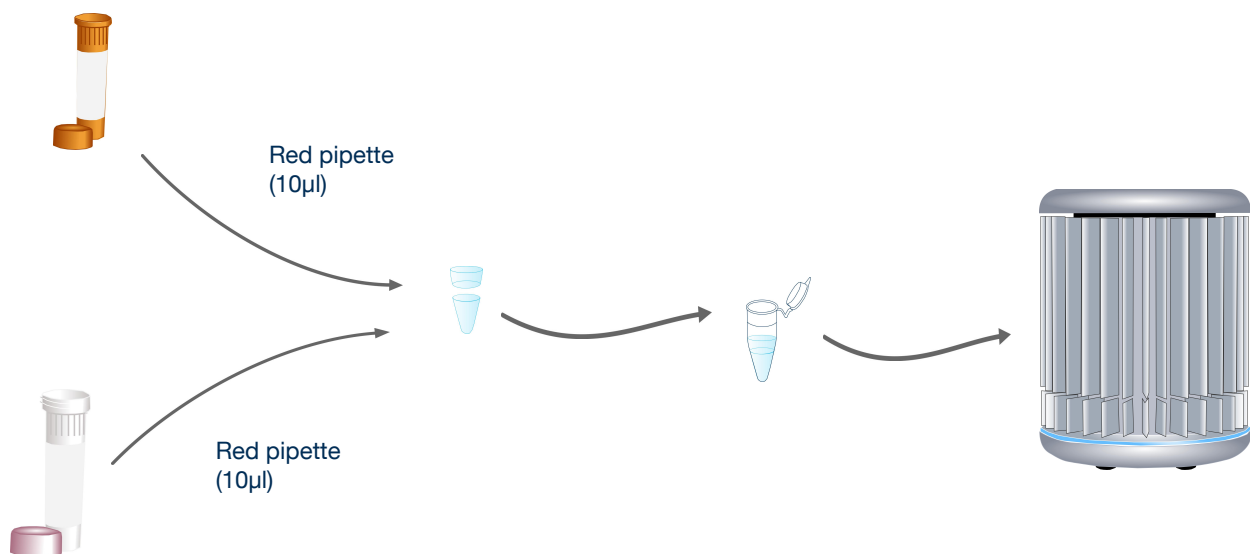


Use the blue pipette to transfer 1000µl (2 x 500µl) of water in to the positive control template tube.

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:

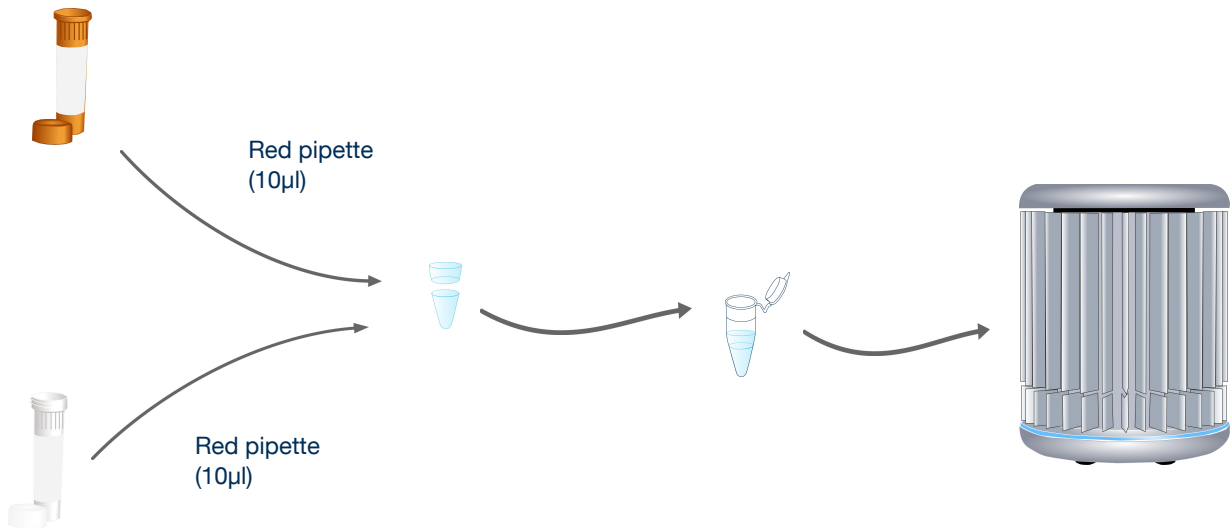
1. It will always test positive so it shows that everything is working as it should be.
2. The q16 software knows how much DNA is present in the positive control. So it can automatically compare your sample of interest with the positive control to calculate the amount of target DNA in your sample.

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



Take great care when setting up your positive control. The positive control DNA has the potential to give you a false positive signal in your other samples. Set them up last after all other sample tubes are closed. Change your pipette tip each time you use it. You may even choose to set up positive controls in a separate room.

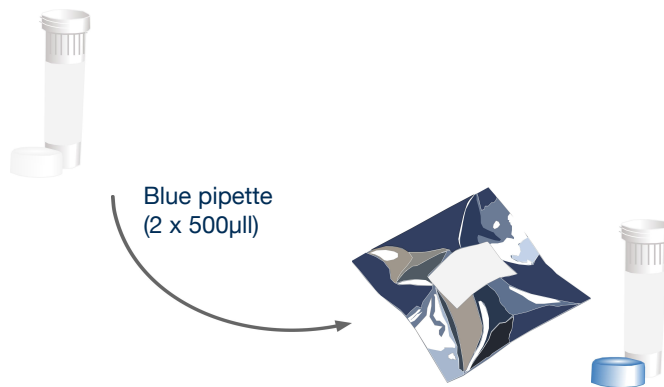
4. Negative control



For each test you will require a negative control. Instead of DNA, water is used. This sample should prove negative thus proving that any of 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 in to account accordingly.

5. Internal extraction control



Use the blue pipette to transfer 1000µl (2 x 500µl) of water in to the internal control template tube.

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

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/percentage.

“Negative”

Explanation

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

“Test Contaminated”

Explanation

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

Solutions

1. Clean your working area using a commercial solution such as “DNA remover” 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 experiment 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/RNA. The result for the Negative Control and the mock sample should be negative indicating that contamination is no longer present.

Preventive action

An ideal lab set-up has a ‘Clean area’ where the test reagents are prepared and a ‘sample area’ where DNA/RNA samples and the Positive Control DNA/RNA are handled. The best workflow involves setting up all the test components 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. The clean area should be decontaminated regularly with DNA remover to keep it clean.

“Sample preparation failed”

Explanation

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

Solutions

1. Check the sample preparation protocol to check for any user errors during preparation and repeat the DNA/RNA extraction.
2. Poor samples can result from overloading the DNA/RNA extraction with too much starting material. Try reducing the amount of starting material and repeat the DNA/RNA extraction.
3. Failing to add the Internal Control Template to your sample during the DNA/RNA extraction process can also lead to a reported result of “DNA/RNA extraction 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 kit; you must add in 5µl of Internal Control Template in to 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 secure. 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. The exact copy number of DNA/RNA present cannot be accurately calculated in this instance. If you quantitative information for your sample then proceed with the solutions below.

Solution

1. Check the DNA/RNA extraction protocol for any user errors during preparation and repeat the DNA/RNA extraction.
2. Poor samples can result from overloading the DNA/RNA extraction with too much starting material. Try reducing the amount of starting material and repeat the DNA/RNA extraction.
3. Failing to add the Internal Control Template to your sample during the DNA/RNA extraction process can also lead to this error. Ensure that this step has not been overlooked or forgotten. If your samples have derived from an archive store or from a process separate from your genesig EASY kit; you must add in 5µl of Internal Control Template in to each 0.5ml of your sample to make it suitable for use on the q16.

“Test failed”

Explanation

The Positive Control is present to show that all aspects of the test are working correctly together. This error message shows that the quality control test has failed and the test as a whole is invalidated. This finding indicates that a problem has occurred in the test set-up part of the experiment and has nothing to do with DNA/RNA extraction.

Solution

1. Check the entire workflow to look for any user errors during test set-up and repeat the test e.g. have the right colour pipettes and solutions been used with the correct tubes?
2. A component of the test may have 'gone off' due to handling errors, incorrect storage or exceeding the shelf life. Open a new kit and run a simplified 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/RNA. If the Positive Control works, the mock sample will now be called as a negative result indicating that all the components of this kit are working correctly.

“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 secure as a positive test. 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 “test sensitivity” column. e.g. If the “test sensitivity” is reported at “1%” then the sample can be reported as “positive test with less than 1% contamination.

Solution

1. 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 reaction.
2. If this is not possible, check the DNA/RNA extraction protocol to check for any user errors during preparation and repeat the DNA/RNA extraction.
3. Poor samples can result from overloading the DNA/RNA extraction with too much starting material. Try reducing the amount of starting material and repeat the DNA/RNA extraction.

“Test failed and is contaminated”

Explanation

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

Solution

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

“Low level of sample DNA ”

Explanation

The test has failed because neither the sample quality nor quantity was high enough. The

Internal Control component identifies the total quantity of sample from which the species percentage is calculated. This signal may be low if the sample is of low quality, or if a low amount of sample was tested. In either case the sample will not be fit for analysis.

Solution

1. Try increasing the concentration of the DNA/RNA sample that is added to the reaction.
2. If this is not possible, check the DNA/RNA extraction protocol to check for any user errors during preparation and repeat the DNA/RNA extraction.
3. Poor samples can result from overloading the DNA/RNA extraction with too much starting material. Try reducing the amount of starting material and repeat the DNA/RNA extraction.

Specificity

The Primerdesign™ genesig® Kit for Enterocytozoon bienersi (E. bienersi) genomes is designed for the in vitro quantification of E. bienersi genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the E. bienersi genome.

The primers and probe sequences in this kit have 100% homology with a broad range of E. bienersi sequences based on a comprehensive bioinformatics analysis.

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 -20°C on arrival. Primerdesign does not recommend using the kit after the expiry date stated on the pack. Once the lyophilized components have been re-suspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

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® E. bienewisi 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 Applied Biosystems 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.

Trademarks

PrimerDesign™ is a trademark of Primerdesign Ltd.

genesig® is a registered trademark of Primerdesign Ltd.