Primerdesign™ Ltd

Enterocytozoon bieneusi

Internal transcribed RNA spacer

genesig® Standard Kit

150 tests

For general laboratory and research use only

DNA testing

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

Introduction to Enterocytozoon bieneusi

Enterocytozoon bieneusi, a fungus of the microsporida 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. bieneusi 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. bieneusi 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.

Specificity

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

The primers and probe sequences in this kit have 100% homology with a broad range of E. bieneusi 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 Contents

- **E. bieneusi specific primer/probe mix (150 reactions BROWN)**
  FAM labelled

- **E. bieneusi positive control template (for Standard curve RED)**

- **RNAse/DNAse free water (WHITE)**
  for resuspension of primer/probe mixes

- **Template preparation buffer (YELLOW)**
  for resuspension of positive control template and standard curve preparation

Reagents and equipment to be supplied by the user

Real-Time PCR Instrument

DNA extraction kit

This kit is recommended for use with genesig EASY DNA/RNA Extraction kit. However, it is designed to work well with all processes that yield high quality DNA with minimal PCR inhibitors.

oasig™ Lyophilised or PrecisionPLUS™ 2 x qPCR Mastermix

This kit is designed to work well with all commercially available Mastermixes. However, we recommend the use of oasig™ or PrecisionPLUS™ 2x qPCR MasterMix.

Pipettors and Tips

Vortex and centrifuge

Thin walled 1.5 ml PCR reaction tubes

Kit storage and stability

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

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.

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 DNA integrity. Always run at least one negative control with the samples. To prepare a negative-control, replace the template DNA sample with RNAse/DNAse free water.

Dynamic range of test

Under optimal PCR conditions genesig® E. bieneusi detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and disclaimers

Trademarks

Principles of the test

Real-time PCR

A E. bieneusi specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the E. bieneusi DNA. 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 real-time PCR platforms.

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 E. bieneusi copy number / CT 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 E. bieneusi gene 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. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources of contamination should first be explored and removed.

Carry-over prevention using UNG (optional)

Carry over contamination between PCR reactions can be prevented by including uracil-N-glycosylase (UNG) in the reaction mix. Some commercial mastermix preparations contain UNG or alternatively it can be added as a separate component. UNG can only prevent carry over from PCR reactions that include deoxyuridine triphosphate (dUTP) in the original PCR reaction. Primerdesign recommend the application of 0.2U UNG per assay with a 15 minute incubation step at 37°C prior to amplification. The heat-labile UNG is then inactivated during the Taq polymerase activation step.

Reconstitution Protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. **Reconstitute the kit components in the RNase/DNase-free water supplied, according to the table below.**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in water	Volume
Pre-PCR pack	
E. bieneusi primer/probe mix (BROWN)	165 µl

3. **Reconstitute the positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
Positive Control Template (RED) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

Real-time PCR detection protocol

1. **For each DNA samples prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
oasig™ or PrecisionPLUS™ 2x qPCR MasterMix	10 µl
E. bieneusi primer/probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of this mix into each well according to your real-time PCR experimental plate set up.**

3. **Prepare DNA templates for each of your samples.**

4. **Pipette 5µl of DNA template into each well, according to your experimental plate set up.**
   For negative control wells use 5µl of RNAse/DNAse free water. The final volume in each well is 20µl.

5. **If a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:**

Component	Volume
oasig™ or PrecisionPLUS™ 2x qPCR MasterMix	10 µl
E. bieneusi primer/probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

6. Preparation of a standard curve dilution series.

1) Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
2) Pipette 10µl of Positive Control Template (RED) into tube 2
3) Vortex thoroughly
4) Change pipette tip and pipette 10µl from tube 2 into tube 3
5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

Standard Curve	Copy Number
Tube 1 Positive control (RED)	2 x 10^5 per µl
Tube 2	2 x 10^4 per µl
Tube 3	2 x 10^3 per µl
Tube 4	2 x 10^2 per µl
Tube 5	20 per µl
Tube 6	2 per µl

7. Pipette 5µl of standard template into each well for the standard curve according to your experimental plate set up.
The final volume in each well is 20µl.

Amplification Protocol

Amplification conditions using oasig™ or PrecisionPLUS™ 2x qPCR MasterMix.

	Step	Time	Temp
	UNG treatment (if required) **	15 mins	37 °C
	Enzyme activation	2 mins	95 °C
50 Cycles	Denaturation	10s	95 °C
	DATA COLLECTION *	60s	60 °C

* Fluorogenic data for the control DNA should be collected during this step through the FAM channel
** Required if your Mastermix includes UNG to prevent PCR carryover contamination

Interpretation of Results

Target	Negative control	Positive control	Interpretation
+ive	-ive	+ive	+ive
+ive	-ive	+ive	+ive
-ive	-ive	+ive	-ive
-ive	-ive	-ive	Experiment fail
+ive	+ive	+ive	Experiment fail