

PrimerDesign™ Ltd

Quantification of *Toxoplasma gondii* genomes

Repeat region

For general laboratory and research use only

Standard Kit





Contents

Introduction to <i>Toxoplasma gondii</i>	3
Specificity	3
Kit Contents	4
Reagents and equipment to be supplied by the user	4
Kit storage	4
Suitable sample material	5
Dynamic range of test	5
Principles of the test	6
Bench side Protocol	8
Amplification Protocol	11
Notices and disclaimers	12
Trademarks	12



Introduction to *Toxoplasma gondii*

Toxoplasma gondii is a species of parasitic protozoa in the genus *Toxoplasma*. [1] The definitive host of *T. gondii* is the cat, but the parasite can be carried by the vast majority of warm-blooded animals, including humans. The causative agent of toxoplasmosis, the disease, is usually minor and self-limiting but can have serious or even fatal effects on a fetus whose mother first contracts the disease during pregnancy or on an immunocompromised human or cat.

Acute stage toxoplasma infections can be asymptomatic, but often gives flu-like symptoms in the early acute stages, and like flu can become, in very rare cases, fatal. The acute stage fades in a few days to months, leading to the latent stage. Latent infection is normally asymptomatic; however, in the case of immunocompromised patients (such as those infected with HIV or transplant recipients on immunosuppressive therapy), toxoplasmosis can develop. The most notable manifestation of toxoplasmosis in immunocompromised patients is toxoplasmic encephalitis, which can be deadly. If infection with *T. gondii* occurs for the first time during pregnancy, the parasite can cross the placenta, possibly leading to hydrocephalus, intracranial calcification, and chorioretinitis, with the possibility of spontaneous abortion or intrauterine death.

Specificity

The *PrimerDesign™ Quantification Kit for Toxoplasma gondii Genomes* is designed for the *in vitro* quantification of toxoplasma. The primers have 100% homology with all reference sequences included in the NCBI database (EF195646.1, AF487550.1, AF146527).



Kit Contents

- Pathogen specific primer/probe mix (150 reactions **BROWN**)
- Pathogen positive control template (for Standard curve **RED**)
- RNase/DNase free water

Reagents and equipment to be supplied by the user

- Real-Time PCR Instrument
- **Mastermix or Mastermix components**
This kit is designed to work well with all commercially available Mastermixes. However, we recommend the use of PrimerDesign 2x *Precision*[™] Mastermix.
- Pipettors and Tips
- Vortex and centrifuge
- Thin walled 1.5 ml PCR reaction tubes

Kit storage

This kit is stable at room temperature. Once the lyophilized components have been re-suspended the kit should be stored at -20°C. Unnecessary repeated freeze/thawing should be avoided. Under these conditions reagents are stable for six months from date of purchase.



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. 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 PrimerDesign pathogen detection kits have very high priming efficiencies of >95% and can detect between 1×10^8 and 1×10^2 copies of target template.



Principles of the test

Real-Time PCR

A pathogen specific primer and probe mix is provided and the target sequence 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 pathogen DNA/cDNA. A fluorogenic probe, is included in the same reaction mixture which consists of an oligonucleotide labeled with a 5`-reporter dye and a downstream, 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 positive control template. This can be used to generate a standard curve of pathogen copy number / CT value. Alternatively the positive control can be used at a single dilution for a qualitative analysis of the samples. Each time the kit is used, at least one positive control reaction must be included on the run. A positive result indicates that the primers and probes for quantification of the target pathogen gene are working properly in your 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.

Negative control

To confirm absence of contamination, 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 or 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 0.2 U 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 (95°C for 10 minutes).



Bench side 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. Barrier 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 according to the table below

To ensure complete reconstitution, vortex each tube thoroughly, allow to stand for 5 minutes and vortex again before use.

Component	Volume
Pre-PCR box	
Primer/Probe mix (BROWN)	165 µl
Post-PCR bottle	
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

1. Prepare a reaction mix according to the table below

Include sufficient reactions for the standard curve wells (8 samples in duplicate) and also the negative control.

Pathogen detection mix

Component	1 reaction
2X Precision™ Mastermix	10 µl
Pathogen primer/probe mix (BROWN)	1.0 µl
RNAse/DNAse Free water	4.0 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 sample DNA templates for each of your samples (suggested concentration 5ng/µl) in RNAse/DNAse free water.

If the concentration of DNA is not known, then dilute your DNA sample reactions 1:20 (10 µl of sample DNA and 190µl of water)

4. Pipette 5µl of diluted 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. Preparation of standard curve dilution series

- 1) Pipette 900 μ l of RNase/DNase free water into 7 tubes and label 2-8
- 2) Pipette 100 μ l of Positive Control Template (**RED**) into tube 2
- 3) Vortex thoroughly
- 4) Change pipette tip and pipette 100 μ l 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)	2×10^7 per μ l
Tube 2	2×10^6 per μ l
Tube 3	2×10^5 per μ l
Tube 4	2×10^4 per μ l
Tube 5	2×10^3 per μ l
Tube 6	2×10^2 per μ l
Tube 7	20 per μ l
Tube 8	2 per μ l

6. Pipette 5 μ l of standard template into each well, according to your experimental plate set up.

The final volume in each well is 20 μ l.



Amplification Protocol

Amplification conditions using PrimerDesign 2X Precision™ MasterMix.

	Step	Time	Temp
	UNG treatment (if required) **	15 mins	37°C
	Enzyme Activation (if required)***	10 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

*** Not all Mastermixes require this enzyme activation step. Follow the manufactures instructions for your mastermix.



Notices and disclaimers

During the warranty period PrimerDesign pathogen 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.

Black Hole Quencher™, "BHQ", "CAL Fluor", "Quasar" and "Pulsar" are registered trademarks of Biosearch Technologies, Inc., Novato, CA. This technology is protected by U.S. and World-wide patents either issued or in application and is licensed and sold under agreement with Biosearch Technologies, Inc. These products are sold exclusively for R&D use by the purchaser. They may not be used for human or veterinary *in vitro* diagnostic (IVD) applications and they may not be re-sold, distributed or re-packaged without express written authorization from Biosearch

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 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. The purchase of Biosearch Technologies products does not, either expressly or by implication, provide a license to use this or other patented technology. Licensing information can be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404 or the Licensing Department at Roche Molecular Systems Inc., 1145 Atlantic Avenue, Alameda, CA 94501."

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.

ABI, ABI PRISM®, GeneAmp® and MicroAmp® are registered trademarks of the Applied 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-La Roche Inc.