

PrimerDesign™ Ltd

Quantification of *Leptospira* genomes

outer membrane protein lipI32

For general laboratory and research use only

Standard Kit





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Introduction to *Leptospira*

Leptospirosis is a bacterial zoonotic disease caused by spirochaetes of the genus *Leptospira*. Spirochaetes are Gram-negative bacteria, which have long, helically coiled cells with distinctive axial filaments, which run lengthwise between the cell membrane and outer membrane. These cause a twisting motion which allows the spirochaete to move about.

Leptospirosis is transmitted by contact with infected blood and other bodily fluids. This can include food or water contaminated with urine from infected animals. Treatment is aimed at suppressing the causative bacteria using antibiotics and fighting possible complications.

In animals the disease most commonly causes damage to the liver and kidneys. Vasculitis can occur, causing edema. Myocarditis, pericarditis, meningitis, and uveitis are also possible sequelae. Vomiting, fever, failure to eat, reduced urine output, unusually dark or brown urine, and lethargy are also indications of the disease.

In humans, leptospirosis can cause many symptoms although in some cases can be asymptomatic. The disease is biphasic with symptoms appearing after a 4-14 day incubation period. Initially the disease presents with flu-like symptoms (fever, chills, myalgias and intense headaches), which resolve leaving the patient briefly asymptomatic before the onset of the second phase. This is characterised by meningitis, liver damage (causing jaundice), and renal failure. Only 1 in 10 cases are classified as severe (i.e. leading to complications), but approximately 5-50% of these cases are fatal.

The causative bacterium has 5 different serovars that are disease causing, with other (less common) infectious strains. Genetically different *leptospira* organisms may be identical serologically and vice versa, leading to difficulties in strain identification.



Specificity

The *PrimerDesign™ Quantification Kit for Leptospira Genomes* is designed for the *in vitro* quantification of all pathogenic serova of *Leptospira* strains. The primers have 100% homology with all NCBI reference sequences included below and therefore have a very broad detection profile

AM937000.I, DQ286418.I, AY776294.I, AY609332.I, AY609329.I, AY609327.I, AY609325.I, AY609324.I, AY609321.I, AY461909.I, AY461908.I, AY461907.I, AY461905.I, AY461903.I, AY461902.I, AY568679.I, AY442332.I, DQ149595.I, AE010300.I, AF366366.I, U89708.I, AF245281.I, AB094436.2, AB094435.2, AB094433.2, AE016823.I, DQ343231.I, AY776293.I, AY609333.I, AY609328.I, AY609326.I, AY461920.I, AY461910.I, AY461906.I, AY461904.I, AY461901.I, AY423075.I, DQ092412.I, AF181553.I, AJ580493.I, AB094437.2, AB094434.2, AY223718.I, AY609323.I, AY461919.I, AY461918.I, AY461917.I, AY461916.I, AY461915.I, AY461914.I, AY461913.I, AY461912.I, AY461911.I, AF181556.I, AF121192.I, CP000350.I, CP000348.I, DQ286417.I, AY609331.I, AY461930.I, AY461899.I, AY461895.I, AY461893.I, AF181554.I, EU526390.I, EU526389.I, EU293442.I, EU293441.I, DQ320625.I, DQ286415.I, AY609330.I, AY609322.I, AY461929.I, AY461900.I, AY461898.I, AY461897.I, AY461896.I, AY568680.I, DQ286416.I, AY461894.I, AY776292.I, AY461928.I, AY461927.I, AY461926.I, AY461925.I, AY461923.I, AY461922.I, AY461921.I, AF181555.I, AY763509.I, AM936999.I, AY461924.I, EU293443.I,



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 but should be stored at -20°C on arrival. Once the lyophilized components have been re-suspended 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 900µl of RNase/DNase free water into 7 tubes and label 2-8
- 2) Pipette 100µl of Positive Control Template (**RED**) into tube 2
- 3) Vortex thoroughly
- 4) Change pipette tip and pipette 100µl 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 µl
Tube 2	2×10^6 per µl
Tube 3	2×10^5 per µl
Tube 4	2×10^4 per µl
Tube 5	2×10^3 per µl
Tube 6	2×10^2 per µl
Tube 7	20 per µl
Tube 8	2 per µl

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

The final volume in each well is 20µl.



Amplification Protocol

Amplification conditions using **PrimerDesign 2X Precision™ MasterMix**.

	Step	Time	Temp
	Enzyme Activation	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



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.

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