MBPCR104 Hi-PCR® Leptospira Probe PCR Kit

Instructions For Use

Description

Leptospirosis is an infection caused by spirochete bacteria called Leptospira. They are bacteria which can be either pathogenic (i.e. having the potential to cause disease in animals and humans) or saprophytic (i.e. free living and generally considered not to cause disease). Humans can get leptospirosis through direct contact with urine from infected animals or through water, soil or food contaminated with their urine. It is most common in warm climates. Symptoms of Leptospirosis range from mild to severe which includes high fever, headache, bleeding, muscle pain, chills, red eyes and vomiting. Without treatment, leptospirosis can lead to kidney and liver damage and even death. Hence, early diagnosis plays an important role for physician to make decision for treatment. Nucleic acid amplification-based assays or Polymerase Chain Reaction (PCR) is one of the confirmatory diagnostic method of Leptospira diagnosis that allows for sensitive and specific detection of Leptospira DNA from clinical samples. Real-Time PCR technique is considerably simple and fast with respect to the standard PCR technique. This technique has been successfully used for the rapid detection and identification of a variety of infectious and non-infectious pathogens and genes.

NOTE: HiMedia's Hi-PCR® Leptospira Probe PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of Leptospira in clinical samples.

Principle

Real-Time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Hi-PCR® Leptospira Probe PCR Kit is designed to detect **23S rRNA gene of Leptospira in FAM channel** with **Internal Control in JOE channel** in a single tube reaction.

Positive Control

This is a control reaction using a known template (target pathogen). A positive control is usually used to check that the PCR conditions have been set up correctly.

Internal Control

This is a heterologous control (Internal Control, IC) sequence that serves as the in-process control to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit. It determines the validity of the tests.

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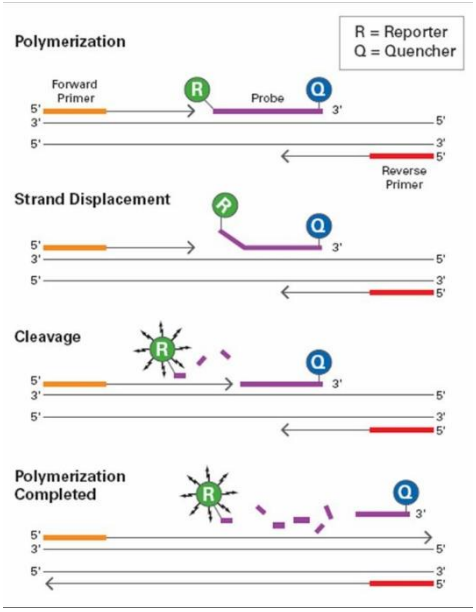
Negative Control

A negative control is needed to ensure that the reagents, equipment, and environment used in the assay is not contaminated. In this reaction, Nuclease free water is used as the template. It is recommended to have minimum 1 reaction of negative control per run.

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-Time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement:When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle,the DNA polymerae cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'- nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

- Fast and simple
- Good sensitivity and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

Sample Source: Blood Samples / Urine Samples / Bacterial cultures

Storage and Shelf life:

The provided kit has a shelf-life of 12 months when stored between -10°C to -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on pack.

Kit Contents:

The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)		
		25R	50R	100R
Hi-Quanti 2X Realtime PCR Master Mix	MBT180	338	675	1325
Leptospira Primer-Probe Mix	DS0763	27	54	106
Internal Control Primer-Probe Mix	DS0378A	27	54	106
Internal Control DNA	DS1096	27	54	106
Leptospira Positive Control	DS0764	25	50	106
Molecular Biology Grade Water for PCR	ML065	200	400	583

***** For a 25 µL PCR reaction

Specimen collection and Handling:

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood and other body fluids. Safety guidelines may be referred in individual safety data sheets.

Sample Preparation

Various Blood Samples, Urine Samples and bacterial culture samples are routinely examined. For extraction and purification of pure bacterial DNA for high yield, perform the nucleic acid purification using HiMedia's HiPurA® Bacterial Genomic DNA Purification Kit (MB505) or HiPurA® Multi-Sample DNA Purification Kit (MB554) as instructed in the protocol.

Materials needed but not provided

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19) & Sealing film (PR18)
- Insta Q Real Time PCR System (Product Code: LA1012 / LA1023 / LA1024 / LA1073 / LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes
- For Blood and / or Urine Sample: HiPurA® Multi-Sample DNA Purification Kit (MB554)
- For Bacterial culture: HiPurA® Bacterial Genomic DNA Purification Kit (MB505)

General Preparation Instructions

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

A. Protocol for PCR Master Mix Preparation

Components	Product code	Volume (µL) to be added for 1R (for a 25 µL reaction)
Hi-Quanti 2X Realtime PCR Master Mix	MBT180	12.5 µL
Leptospira Primer-Probe Mix	DS0763	1 µL
Internal Control Primer-Probe Mix	DS0378A	1 µL
Internal Control DNA	DS1096	1 µL
Molecular Biology Grade Water for PCR	ML065	4.5 µL
Template DNA / Positive Control / Negative Control	-	5 µL
Total volume	-	25 µL

Centrifuge the tube briefly at 6000 rpm for about 10 seconds. Place the tubes in Real-Time PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

B. Recommended PCR program

1. Initial denaturation : 95°C for 10 minutes
2. Denaturation : 95°C for 15 seconds
3. Annealing : 55°C for 30 seconds (Sampling)
   Sampling : FAM/JOE

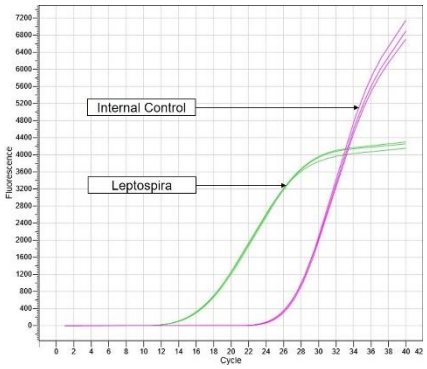
   } No. of cycles: 40
4. Hold : 4°C for ∞

C. Data Analysis

The following conditions should be met for a valid diagnostic test:

Control	Detection channel	
	FAM (Leptospira)	JOE (Internal Control)
Positive Control	+	+
Negative Control	-	+

D. Amplification Data



Sr. No.	Sample	C_t value
1.	Leptospira Positive Control	15.84
2.	Internal Control	27.67

Note: Image representing probe based Real-Time amplification of *Leptospira* DNA with Ct values using HiMedia's Hi-PCR® Leptospira Probe PCR Kit (Ct values provided in table are for representation). The results completely depend upon sample types.

E. Data Interpretation

Ct value	Result
≤ 35	Detected (+)
> 35 or N/A	Not detected (-)

Target		Result Interpretation
Leptospira (FAM)	**Internal Control (JOE)**	
Ct value ≤ 35	Ct value ≤ 35*	Positive for Leptospira
No Ct or > 35	Ct value ≤ 35	Negative for Leptospira
No Ct or > 35	No Ct or > 35	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

*The presence or absence of a signal in the JOE channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

Limit of Detection (LoD)

Sensitivity for the Hi-PCR® Leptospira Probe PCR Kit was conducted using Genomic DNA from *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130 (BAA-1198D-5™). The detectable limit of the Hi-PCR® Leptospira Probe PCR Kit was determined to be 10 copies/µL.

Warning

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use.

Precautions

Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

Performance and Evaluation

Each lot of HiMedia's Hi-PCR® Leptospira Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Leptospira Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of DNA using agarose gel electrophoresis.
			2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
2.	Variability between replicates	Error in reaction set-up	Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a real-time PCR instrument.
		Pipetting error	C_t values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.
4.	No signal with positive controls	Incorrect programming of the temperature profile of the thermocycler	Compare the temperature profile to the manual.

Safety Information

HiMedia's Hi-PCR® Leptospira Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

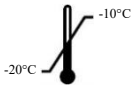
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Symbols:

	Manufacturer		Do not use if package is damaged
	Authorized representative in the European Community		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		In vitro diagnostic medical device
	Batch code		CE marking of conformity
	Catalogue number		

Authorized representative (AR) Address :

	**AR Experts B.V.** Boeingavenue 209, 1119 PD, Schiphol-Rijk, The Netherlands

Identification No.: PIMBPCR104
Rev.No.: 09
Date of Issue: 2026-01