## MBPCR140 Hi-PCR® Legionella species Probe PCR Kit

*Legionella* are thin, Gram-negative, non-encapsulated, obligate aerobic and sporeless bacilli with a single, polar flagellum. *Legionella* spp. are important waterborne pathogens that are normally transmitted through aerosols. They are widespread in natural water sources and often colonizes (become established) in manufactured water systems. Certain species of *Legionell*a like *Legionella pneumophila* are often strongly associated with asymptomatic infections (Legionnaires' disease) or produce mild cough, sore throat and fever (Pontiac fever) that goes away by itself in 2 to 5 days. The term "legionellosis" may be used to refer to as Legionnaires‘ disease, Pontiac fever or extra pulmonary infection. It is a relatively common cause of community-acquired and nosocomial pneumonia in adults. Significant mortality rates among the elderly and patients with severe underlying disease may occur as a result of infection with this pathogen. Diagnostic delay may also result in increased mortality. Therefore, rapid tests such as direct fluorescent-antibody stains and urinary antigen assays, have been developed. Although useful, these assays have sensitivities less than 100%.

**NOTE**: HiMedia’s Hi-PCR® Legionella species Probe PCR Kit is for *in-vitro* use only.

### Intended Use
Recommended for sensitive and specific detection of *Legionella* species in clinical and environmental 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® Legionella species Probe PCR Kit is designed to detect the **23S-5S ribosomal intergenic spacer region of *Legionella* in FAM channel** with **Internal Control in HEX channel** in a single tube reaction. The kit allows sensitive and specific detection of *Legionella* spps. 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 primers have been designed properly and the PCR conditions have been set up correctly.

### Internal Control
This is a control sequence which is amplified in the same reaction tube along with the target sequence (target species) but detected with a different primer (i.e. Multiplex PCR). An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.



ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED CE

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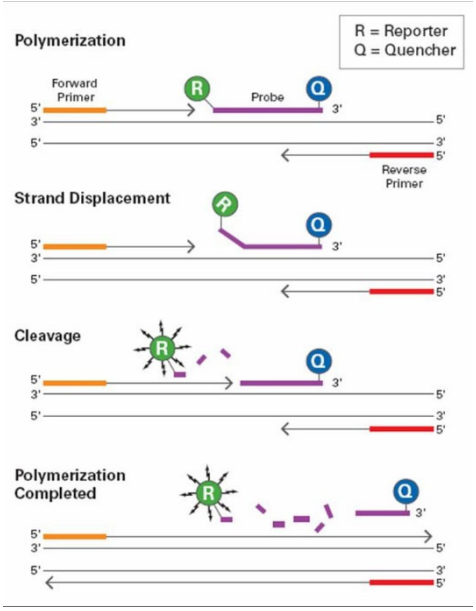
**Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR**




**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:** Bacterial culture / Blood sample / Water sample

**Storage and Shelf life**

The provided kit has a shelf-life of 12 months when stored between -10° 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 |
| Hi-Quanti 2X Realtime PCR Master Mix | MBT180 | 338 | 675 |
| *Legionella* spps. Primer-Probe Mix | DS0638 | 27 | 54 |
| Internal Control Primer-Probe Mix | DS0618A | 27 | 54 |
| Internal Control DNA | DS0619 | 27 | 54 |
| Positive Control (Legionella DNA) | DS0359A | 25 | 50 |
| Molecular Biology Grade Water for PCR | ML065 | 200 | 400 |

***** For a 25 µL PCR reaction

**Warning and Precautions**

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

**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.

**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.

**A. Protocol for PCR Master Mix Preparation**

| Components | Product Code | Volume to be added for 1R (for a 25 µL reaction) |
|---|---|---|
| Hi-Quanti 2X Realtime PCR Master Mix | MBT180 | 12.5 µL |
| Legionella spps. Primer-Probe Mix | DS0638 | 1 µL |
| Internal Control Primer-Probe Mix | DS0618A | 1 µL |
| Internal Control DNA | DS0619 | 1 µL |
| Molecular Biology Grade Water for PCR | ML065 | 4.5 µL |
| Template DNA / Positive Control / Negative Control | - | 5 µL |
| Total volume | - | Upto 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).

**NOTE: (Optional) – The user can also set up an additional PCR reaction containing 5 µL Positive Control (DS0359A) for PCR in a separate tube.**

**B. Recommended PCR program**

| | | | | |
|---|---|---|---|---|
| 1. | Initial denaturation | : | 95°C for 10 minutes | |
| 2. | Denaturation | : | 95°C for 15 seconds | No. of cycles: 40 |
| 3. | Annealing | : | 60°C for 30 seconds (Sampling) | |
| | Channels | : | FAM/HEX | |
| 4. | Hold | : | 4°C for ∞ | |

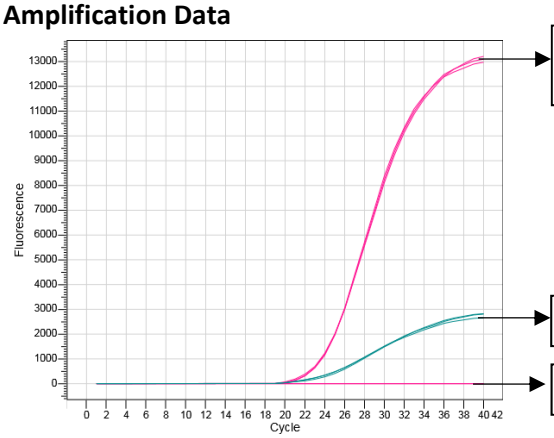
**Data Analysis**

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

| Control | Detection channel | |
|---|---|---|
| | **FAM (*Legionella* spps.)** | **HEX (Internal Control)** |
| **Positive Control** | +ve (≤ 35 cycles) | +ve (15 – 35 cycles*) |
| **Negative Control** | - ve (No Ct or > 35 cycles) | +ve (15 – 35 cycles*) |

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

**Amplification Data**




| Sr. No. | Sample | $C_t$ value |
|---|---|---|
| 1. | *Legionella* spps. positive control | 24.31, 24.26 |
| 2. | Internal Control | 23.69, 23.7 |
| 3. | Negative control | N/A |

**Image representing amplification plot of *Legionella* spps. DNA with Ct values using HiMedia's Hi-PCR® Legionella species Probe PCR Kit. The results completely depend upon sample types.**

**Data Interpretation**

| Detection Channel | | |
|---|---|---|
| **FAM (*Legionella* spps.)** | **HEX (Internal Control)*** | **Result Interpretation** |
| + | + | Positive for *Legionella* spps. |
| - | +/- | Negative for *Legionella* spps. |
| - | - | PCR inhibition or reagent failure.<br>Repeat PCR or repeat extraction from original sample |

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

**Performance and Evaluation**

Each lot of Hi-PCR® Legionella species Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

**Quality Control**

Each lot of HiMedia's Hi-PCR® Legionella species 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.<br>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® Legionella species 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.

| | |
|---|---|
| IVD | In vitro diagnostic medical device |
| CE | CE Marking |
| -10°C -20°C | Storage temperature |
| | Do not use if package is damaged |
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