MBPCR006 Hi-PCR® Legionella pneumophila Semi-Q PCR Kit

Description:

Legionella pneumophila is the most common pathogenic species of the 42 recognized *Legionella* species. 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%. Nucleic acid amplification assays have been shown to be useful for detection of Legionella. The genes that encode the 5s and 16s ribosomal subunits have been shown to contain signature sequences that are useful for identification of *L. pneumophila* and a variety of organisms.

NOTE: The Hi-PCR® Legionella pneumophila Semi-Q PCR Kit is for *in vitro* use only.

Intended Use:

HiMedia's Hi-PCR® Legionella pneumophila Semi-Q PCR Kit is a qualitative conventional PCR kit which allows amplification of Legionella pneumophila specific rRNA **(901 bp)** gene, using specific primers. The amplified target is detected using agarose gel electrophoresis.

Principle:

The Hi-PCR® Legionella pneumophila Semi-Q PCR Kit is designed to detect the specific gene regions of rRNA **(901 bp)** gene *for Legionella pneumophila* in various food sources, cells, environmental and clinical samples. Conventional PCR testing can provide rapid, sensitive and specific detection of *Legionella* species and *L. pneumophila*. This kit also contains **Internal control** and **Positive control.**

Internal control: This is a control sequence which is amplified in the same reaction tube along with the target sequence (target pathogen) 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.

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.

Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of genes. The three steps of a successful PCR reaction include Denaturation, Annealing and Extension. The double-stranded DNA melts and forms single stranded DNA at high temperature (Denaturation). Sequence-specific primers bind to the target sequence on single-stranded DNA at lower temperature (Annealing). Taq DNA Polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (Extension). These 3 steps of PCR are usually repeated between 30 to 40 times in each PCR assay.

Gel electrophoresis is used to analyze the amplification of desired gene region for target pathogen based on separation of DNA fragments according to their size.

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Features:

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

Sample Source: Clinical & Food samples.

Storage:

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 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 sensitivity of the assay. HiMedia does not recommend using the kit after the expiry date stated on pack.

Kit Contents:

The provided PCR contains:

Components	Product codes	Reagents provided for (reactions)* (µL)	
		25R	**50R**
2X PCR TaqMixture	MBT061	675	1350
Primer Mix for *L. pneumophila*	DS0130	54	108
Primer Mix for Internal Control (285 bp)	DS0223	54	108
Molecular Biology Grade Water for PCR	ML065	490	980
6X Gel Loading Buffer	ML015	54	108
100 bp DNA Ladder	MBT049	20	40
Positive control (*L. pneumophila* DNA)	DS0359	5	10
Internal Control DNA	DS0123	27	54

***** For a 50µl 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 food and environmental samples, clinical materials, cultured bacteria and human fecal specimens are routinely examined. For preparation of bacterial DNA, perform the nucleic acid purification using HiMedia's HiPurA® Bacterial DNA Purification Kit (MB505) as described 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)

- Thermal Cycler (Product Code: LA948 / LA949 / LA950 / LA1006 / LA1015/ LA1059 / LA1060 / LA1066)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes

General Preparation Instructions:

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

A. Protocol for PCR Master Mix Preparation

Perform PCR reactions for each DNA sample as per the following table:

Components	Recommended volume to be added per reaction (µL)
2X PCR TaqMixture **(MBT061)**	25 µL
Primer Mix for *L. pneumophila* **(DS0130)**	2 µL
Primer Mix for Internal Control (285 bp) **(DS0223)**	2 µL
Template DNA	2 µL
Internal Control DNA **(DS0123)**	1 µL
Molecular Biology Grade Water for PCR **(ML065)**	Up to 50 µL

NOTE: (Optional) – The user can also set up an additional PCR reaction containing 1µL of Positive control DNA (provided) in a separate tube.

Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below). Interpret the data using Agarose Gel Electrophoresis.

B. Recommended PCR program:

1	Initial denaturation	:	94°C for 10 minutes	No. of cycles: 1
2	Denaturation	:	94°C for 30 seconds	No. of cycles: 30
3	Annealing	:	60°C for 30 seconds	
4	Extension	:	72°C for 45 seconds	
5	Final Extension	:	72°C for 10 minutes	No. of cycles: 1

C. After amplification, the products may be kept at 4°C overnight or frozen at –20°C for long-term storage.

D. Legionella PCR Assay Results Interpretation:

- For analysis of the PCR data, load 10 µl of amplicon on a 1.5% Agarose gel along with 1µl of 6X Gel Loading Buffer (ML015).
- Load 4 µl of 100 bp DNA ladder (MBT049) in separate well.

E. EtBr-staining to check results:

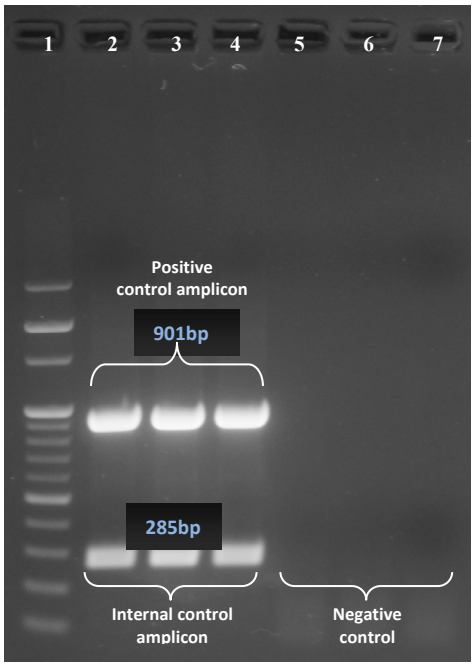
- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 min

- Confirm the expected amplicon size comparing with 100 bp DNA marker.

F. Quality Control:

Each lot of HiMedia's Hi-PCR® Legionella pneumophila Semi-Q PCR Kit is assayed for contaminating endonucleases, exonucleases and non-specific DNase activities. Functionally tested in DNA amplification.

G. Amplification Data:



Lane no.	Samples
1	100 bp ladder
2,3,4	Positive control amplicon of *L. pneumophila* (901 bp) with internal control amplicon (285 bp)
5,6,7	Negative control

Gel image representing amplification of rRNA gene region using target sample of *L . pneumophila* with positive control (901 bp) and internal control (285bp).

Warning

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

Precautions

Read the procedure carefully before starting the experiment. 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.

Performance and Evaluation

Each lot of HiMedia's Hi-PCR® Legionella pneumophila Semi-Q PCR Kit is tested against predetermined specifications to ensure consistent product quality.

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 large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques 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.

Safety Information

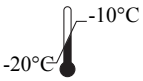
The Hi-PCR® Legionella pneumophila Semi-Q PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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 off 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
	Storage temperature
	Do not use if package is damaged
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