MBPCR263 Hi-PCR® Influenza Multiplex Probe PCR Kit

Instructions For Use

Description

Influenza (commonly referred to as flu), a highly infectious disease known to cause an acute respiratory illness has afflicted humans since ancient times. Influenza viruses are a major cause of morbidity and mortality worldwide. The virus belongs to the family of Orthomyxoviridae that exist in three types, designated as influenza A, Influenza B and Influenza C. Influenza A virus infects humans and many other mammalian species including horses and swine and a wide variety of avian species while Influenza B virus naturally infects only humans. On the contrary, Influenza C virus rarely infects humans. In April 2009, a novel strain of swine origin influenza A H1N1 virus emerged in humans which caused a significant loss of lives across the globe. Influenza A and Influenza B viruses are responsible for annual epidemics and irregular pandemics in humans. The grave threat posed by these emerging and re-emerging influenza viruses requires rapid laboratory detection. Real-time reverse-transcription polymerase chain reaction (RT-PCR) is considered as a gold standard method for influenza surveillance and diagnosis due to its high specificity, sensitivity and rapid detection. HiMedia has developed a Multiplex Reverse Transcriptase Real-Time PCR kit which enables the clinicians and public health laboratories to quickly diagnose influenza infection in a single tube.

NOTE: Hi-PCR® Influenza Multiplex Probe PCR Kit is for in-vitro use only.

Intended Use

Hi-PCR® Influenza Multiplex Probe PCR Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures. The kit is recommended for sensitive and specific detection of influenza virus in clinical samples.

Product Description

Hi-PCR® Influenza Multiplex Probe PCR Kit includes primer-probe sets specific to detect RNA from the three Influenza viruses: A, B & H1N1 pdm 09. In addition, an internal control (IC) for testing successful reactions. The Kit also provides synthetic positive controls for validity of the test.

Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to ensure proper and intended functioning of all the reagents and is recommended to be used in every run to assess optimal performance.

Negative Control

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

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

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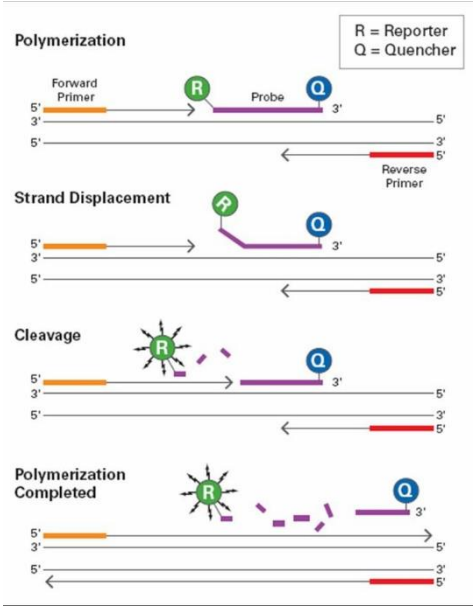
Internal Control

This is a control sequence that should amplify in all clinical samples which indicates the presence of sufficient RNA from human gene indicating the specimen is of acceptable quality. An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

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 target 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. This kit is designed to detect specific RNA targets from H1N1 pdm 09 virus in JOE channel, Influenza A virus (Inf A) in ROX channel and Influenza B virus (Inf B) in Cy5 channel. The internal control is FAM channel.

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 – Real-Time PCR in less than 1.5 hours
- Highly sensitive and specific for detection of Influenza virus
- Includes all reagents and controls
- Synthetic positive controls provided for validity of the test
- Guaranteed reproducible results

Types of Specimens: RNA sample extracted from Bronchoalveolar lavage, tracheal aspirate, sputum, nasopharyngeal swab, oropharyngeal swab, nasopharyngeal wash/aspirate or nasal aspirate using a standard viral RNA extraction kit. Before extraction, specimens can be stored at 4°C up to 72 hours after collection. If any delay is expected in extraction, it is recommended to store specimens at -70 °C or lower. After extraction, store the extracted RNA samples at -20°C for short period storage and -70°C or lower for long period storage.

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 other body fluids. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -15°C to -25°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. This kit can be used for maximum 6 repeats of freezing and thawing. Degradation of sample RNA 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)*		
		25R	50R	100R
FLU Master Mix	DS1218	0.292 mL	0.567 mL	1.134 mL
FLU Primer-Probe Mix	DS1216	0.033 mL	0.063 mL	0.126 mL
FLU Positive Control	DS1217	0.052 mL	0.1 mL	0.2 mL
Water	DS0440	0.052 mL	0.1 mL	0.2 mL

***For a 20 µL PCR reaction**

Materials needed but not provided: All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q48® M4: Real time PCR System, 48 well block, 4 channels	LA1023
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Tubes, plates, and other consumables	
Varivol II Micropipette-10 (Capacity: 0.5 to 10 µl)	LA611
Varivol II Micropipette-100 (Capacity: 10 to 100 µl)	LA615
Varivol II Micropipette-1000 (Capacity: 200 to 1000 µl)	LA614
Barrier Tips, Maximum capacity 10 µl	LA749/LA749A
Barrier Tips, Maximum capacity 200 µl	LA751/LA751A
Barrier Tips, Maximum capacity 1000 µl	LA859/LA859A
Micro Centrifuge Tube - B	PW146
Micro Centrifuge Tube-C	PW147
8-strip tubes & optically clear flat caps for PCR	PR17
PCR Tubes, 0.2 mL; PCR Plates	PW1255/ PR2/PR3/PR19
Polypropylene Sealing film	PR21
Optical Sealing film	PR18
RNase Kil™	ML162

Kit Compatibility with Real-Time PCR systems:

Hi-PCR® Influenza Multiplex Probe PCR Kit contains fluorophores compatible to:

Real-Time PCR system	Company	Dye 1 (IC)	Dye 2 (H1N1)	Dye 3 (Inf A)	Dye 4 (Inf B)
Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48®	HiMedia Laboratories Pvt. Ltd.	FAM	JOE	ROX	Cy5
QuantStudio™ 5	Applied Biosystems	FAM	VIC	ROX	Cy5
Applied Biosystems 7500	Applied Biosystems	FAM	JOE	ROX	Cy5
BioRad CFX96	Bio-Rad Laboratories, Inc.	FAM	HEX/VIC	Texas Red	Cy5
Rotor-Gene® Q/Corbett Rotor-Gene® 6000	QIAGEN	Green	Yellow	Orange	Red
Roche LightCycler® 96	Roche	FAM	HEX/VIC	Texas Red	Cy5
AriaMx	Agilent	FAM	HEX	ROX	Cy5
Alta RT-96E/96S	Athenese-Dx Private Limited	FAM	VIC, HEX, TET, JOE	ROX, Texas Red	Cy5

Note: Ensure that the Real-Time PCR system is calibrated for dyes and is maintained according to the manufactureŕs instructions and recommendations.

Warning and Precautions

Certified for *in-vitro* diagnostics. 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.

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.

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

1. In the "Master mix Preparation" area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for several seconds. Keep on ice for later use.
2. Based on the number of specimens to be tested (N), including the PTC and NTC, calculate the volume of the components to be added as N* volume of 1X

Components	Volume to be added for 1X (for a 20 µL reaction)
FLU Master Mix	10.8 µL
FLU Primer-Probe Mix	1.2 µL
Test – Extracted Sample RNA	8.0 µL
Total volume	**20 µL**

3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the reaction system. After all the reagents are added, mix them thoroughly and centrifuge for several seconds.
4. Load 12 µL of master mixture into the 0.1/0.2 mL PCR reaction plate/strips, compatible to the instrument to be used.
5. Add 8 µL of nuclease free water to the negative control tube.
6. In the "Nucleic acid handling" area, add 8 µL of FLU Positive Control and extracted test RNA into the respective tubes.
7. Tightly cap the strips or seal the plate using an optically clear adhesive film.
8. Briefly, spin the strips/tubes to settle the reagent to the bottom of the tube.
9. Place the plate/strips in Real-time PCR machine and set the PCR program.

B. Recommended PCR program:

1.	cDNA Synthesis	:	55°C for 20 minutes	
2.	Initial denaturation	:	95°C for 02 minutes	
3.	Denaturation	:	95°C for 15 seconds	
4.	Annealing	:	55°C for 45 seconds (Plate Read)	No. of cycles: 45
5.	Extension	:	72°C for 15 seconds	
	Channel	:	FAM/JOE/ROX/Cy5	

Note: For ABI and QuantStudio systems select the passive reference dye and Quencher as "NONE".

C. Data Analysis

When the following controls met the stated requirement the PCR run is considered valid, and the specimens can be considered for interpretation.

Control	Target			
	H1N1 (JOE)	Inf A (ROX)	Inf B (Cy5)	IC (FAM)
Positive Template Control (PTC)	+	+	+	+
Negative Template Control (NTC)	-	-	-	-

D. Cutoff

1. All clinical samples should exhibit IC amplification at or below 37 Ct value, thus suggesting the presence of sufficient RNA from human gene indicating the specimen is of acceptable quality. If the samples are from non-human origin (animal/avian species) or if the RNA is extracted from cell culture supernatant sample, usually such samples exhibit low or no amplification for IC gene.
2. For the interpretation of target gene, follow the below mentioned chart.

Target gene	Detected (+)	Not Detected (-)
H1N1	Ct value ≤ 36	Ct value ≥ 37 or ≤ 45
Inf A	Ct value ≤ 37	Ct value ≥ 38 or ≤ 45
Inf B	Ct value ≤ 36	Ct value ≥ 37 or ≤ 45
IC	Ct value ≤ 37	Ct value ≥ 38 or ≤ 45

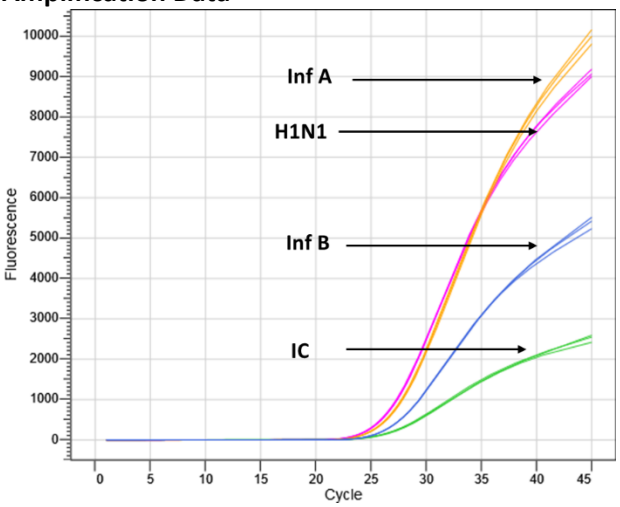
E. Data Interpretation

Targets				Assay Interpretation
H1N1 (JOE)	Inf A (ROX)	Inf B (Cy5)	IC (FAM)	
+	+	-	+	influenza A (H1N1) pdm 09 Positive
-	+	-	+	Influenza A Positive
-	-	+	+	Influenza B Positive
+	+	+	+	influenza A (H1N1) pdm 09 & Influenza B co-infection
-	+	+	+	Influenza A & Influenza B co-infection
-	-	-	+	Negative for influenza virus
-	-	-	-	Invalid test. Repeat extraction or obtain a new specimen for analysis

Note

- Positive results are indicative of the presence of Influenza RNA. However, clinical correlation along with patient history is necessary to determine patient infection status. Positive results also do not rule out bacterial infection or co-infection with other viruses.
- Negative results must be combined with clinical observations and patient history. Negative results do not exclude influenza infection and should not be used as the sole basis for patient management.
- Little is known about epidemiology and outcomes of influenza co-infection. Such infections should not be ruled out. However, more studies are needed to assess the effect of influenza co-infection in clinical outcomes.

Amplification Data



Sr. No	Target	Ct value	
		PTC	NTC
1	IC	26.49	--
2	H1N1	26.83	--
3	Inf A	28.49	--
4	Inf B	27.33	--

Note: Image representing probe based Real-Time amplification of pH1 gene, IC, Inf A and Inf B (Ct values provided in table are for representation).

Performance Evaluation

Limit of Detection (LoD) - Analytical Sensitivity

The analytical sensitivity was defined as the lowest concentration of the target that could be reliably detected with 95% confidence. The analytical sensitivity for the Hi-PCR® Influenza Multiplex Probe PCR Kit was conducted using ATCC Synthetic RNA with negative respiratory samples of influenza virus as clinical matrix. The preliminary LoD of each target was determined by testing a 10-fold dilution series in triplicates per concentration, and then confirmed with 20 replicates of the concentration determined to

be the LoD. The data revealed that Hi-PCR® Influenza Multiplex Probe PCR Kit detects 2 copies/µL of influenza A (H1N1) pdm 09, 4 copies/µL of Influenza A and 2 copies/µL of Influenza B RNA with a confidence ≥95%.

Inclusivity - Analytical Sensitivity

In-silico analysis for the assessment of inclusivity for the Hi-PCR® Influenza Multiplex Probe PCR Kit was conducted by mapping the primers and probes against all the available sequences in GenBank. The Hi-PCR® Influenza Multiplex Probe PCR Kit targets 100% of the known influenza A (H1N1) pdm 09, Influenza A and Influenza B strains.

Cross-reactivity - Analytical Specificity

Wet testing analysis was performed against the recommended list of organisms for respiratory samples. No cross-reaction was observed with any strains mentioned below.

Severe acute respiratory syndrome-related coronavirus 2, SARS-CoV-2	Human rhinovirus 16
Human coronavirus 229E	Human respiratory syncytial virus
Human coronavirus HKU1	*Candida albicans*
Human coronavirus NL63	*Legionella pneumophila*
Human coronavirus OC43	*Mycobacterium tuberculosis*
Human metapneumovirus (hMPV)	*Pseudomonas aeruginosa*
Human parainfluenza virus 1	*Staphylococcus epidermidis*
Human parainfluenza virus 2	*Escherichia coli*
Human parainfluenza virus 3	*Staphylococcus aureus*
Human adenovirus 1 Adenoid 71	*Klebsiella pneumoniae*
Enterovirus 68 strain Fermon	

In addition, a separate *in-silico* analysis was performed (as described above) for the pathogens recommended by the US FDA but not available for wet testing (see list below). The Hi-PCR® Influenza Multiplex Probe PCR Kit shows 100% specificity to influenza A (H1N1) pdm 09, Influenza A and Influenza B strains only.

Human parainfluenza virus 4	*Streptococcus pneumoniae*
MERS-coronavirus	*Streptococcus pyogenes*
Cytomegalovirus	*Mycoplasma pneumoniae*
Epstein–Barr virus	*Bordetella pertussis*
Measles morbillivirus	*Corynebacterium*
Mumps orthorubulavirus	*Lactobacillus*
Chlamydia pneumoniae	*Neisseria meningitidis*
Hemophilus influenzae	*Streptococcus salivarius*

Evaluation

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

Quality Control

Each lot of Hi-PCR® Influenza Multiplex Probe PCR Kit is assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. Functionally tested in amplification.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Use freshly prepared RNA 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 thermal cycler	Compare the temperature profile to the manual.

Safety Information

Hi-PCR® Influenza Multiplex 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.

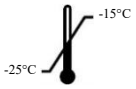
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures while disposing the infectious materials. 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		

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