MBPCR255 Hi-PCR® COVID-19 Triplex Probe PCR Kit

Description

A series of severe unexplained viral pneumonia cases of unknown cause emerged in Wuhan, Hubei, China, in December 2019 was later identified as coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Coronaviruses are enveloped viruses with a positive-sense single-stranded RNA genome and a nucleocapsid of helical symmetry with a characteristic club-shaped spikes on the surface. They are highly diverse due to constant mutations and recombination. There are about 40 different varieties of coronavirus distributed mainly into 5 genera. SARS-CoV-2 is a β coronavirus, subgenus Sarbecovirus, 150-200nm in diameter with a genome size of about 30 Kb. In response to the novel coronavirus (SARS-CoV-2) outbreak, HiMedia has developed a multiplex Reverse Transcriptase Real-Time PCR kit which enables the clinicians and public health laboratories to quickly diagnose COVID-19 infection. This Real-Time PCR kit is a fast, highly sensitive multiplex diagnostic solution for detection of RNA from the SARS-CoV-2 virus.

NOTE: Hi-PCR® COVID-19 Triplex Probe PCR Kit is for *in-vitro* use only.

Intended Use

Hi-PCR® COVID-19 Triplex 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 SARS-CoV-2 in clinical samples.

Product Description

Hi-PCR® COVID-19 Triplex Probe PCR Kit includes primer-probe sets specific to Nucleoprotein (N) gene and RNA-dependent RNA polymerase (RdRp) gene and internal process control. 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 SARS-CoV-2 RNA. In this reaction, Nuclease free water is used as the template. It is recommended to have minimum 1 reaction of negative control per run.

Internal Control

This is a control sequence that should amplify in all clinical samples which indicates the presence of sufficient RNA from human endogenous gene i.e. human L35a Ribosomal Protein gene (RPL35A) 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.

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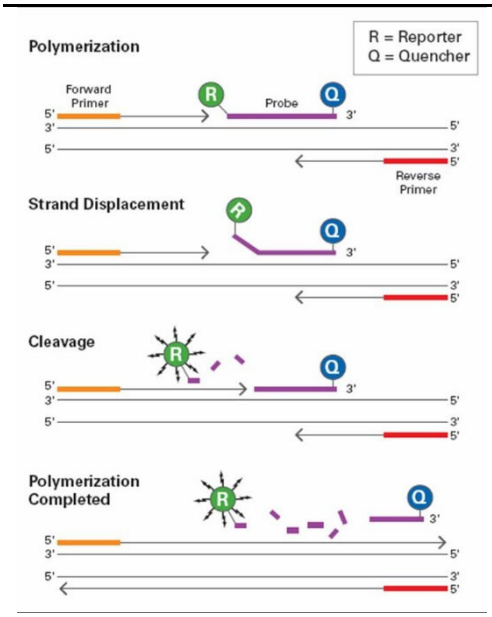
Principle

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. This kit is designed to detect the Nucleoprotein (N) gene and RNA-dependent RNA polymerase (RdRp) gene specific for SARS-CoV- 2 in FAM and TexasRed channels respectively. The RPL35A gene serves as an internal control in JOE 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 – samples to results within 2 hours
- Highly sensitive and specific for SARS-CoV-2 detection
- Includes all reagents and controls
- Synthetic positive controls provided for validity of the test
- Guaranteed reproducible results

Types of Specimen: 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 -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. 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)*	
		50R	100R
2X One Step Buffer Mix	DS1086	0.51 mL	1.02 mL
One-step RT Enzyme Mix	DS1087	0.042 mL	0.084 mL
SARS-CoV-2 Primer-Probe Mix	DS1088	0.078 mL	0.156 mL
SARS-CoV-2 Positive Control	DS1089	0.075 mL	0.15 mL
Water	DS0440	0.05 mL	0.1 mL

***For a 20 µL PCR reaction**

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

Product name	Product Code
Dry Heat Bath	
Wee Dry™	LA1115
Hiper® Temp Shaker	LA1098
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/PR22/PR23
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® COVID-19 Triplex Probe PCR Kit contains fluorophores compatible to:

- Insta Q96® - 6.0 Real time PCR System (HiMedia Laboratories Pvt. Ltd.)
- Insta Q96® Plus Real time PCR System (HiMedia Laboratories Pvt. Ltd.)
- Insta Q96® Real time PCR System (HiMedia Laboratories Pvt. Ltd.)
- Insta Q48® M4 Real time PCR System (HiMedia Laboratories Pvt. Ltd.)
- CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.)
- Applied Biosystems™ 7500 Real-Time PCR System (Applied Biosystems)
- QuantStudio™ 3/5 Real−Time PCR Instrument (Applied Biosystems)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- Corbett Rotor-Gene® 6000 (QIAGEN)
- LightCycler® 480 Instrument II (Roche)
- AriaMx Real-Time PCR System (Agilent)

Note: Ensure that the Real-Time PCR system is calibrated for FAM dye, JOE/HEX dye and Texas Red/ROX dye, and is maintained as according to the manufactureŕs instructions and recommendations.

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/ Personal protective equipment (PPE)/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)**
2X One Step Buffer Mix	10 µL
One Step RT Enzyme Mix	0.8 µL
SARS-CoV-2 Primer-Probe Mix	1.5 µL
Positive Control/Test Sample/Negative Control	7.5 µL
Water	0.2 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 12.5 µL of master mixture into the 0.1/0.2 mL PCR reaction plate/strips, compatible to the instrument to be used, add 7.5 µL of the negative control.
5. In the "Nucleic acid handling" area, add 7.5 µL of SARS-CoV-2 Positive Control and extracted test RNA into the plate/strip.
6. Tightly cap the strips or seal the plate using an optically clear adhesive film.
7. Briefly, spin the strips/tubes to settle the reagent to the bottom of the tube.
8. Place the plate/strips in Real-time PCR machine and set the PCR program.

B. Recommended PCR program

1.	cDNA Synthesis	:	50°C for 15 minutes	
2.	Initial denaturation	:	95°C for 30 seconds	
3.	Denaturation	:	95°C for 03 seconds	No. of cycles: 45
4.	Annealing	:	58°C for 30 seconds (Plate Read)	
	Channel	:	FAM/JOE#/TexasRed#	

#for instruments not calibrated for JOE, HEX can be used. #for instruments not calibrated for Texas Red, ROX can be used.

C. Data Analysis

The following conditions should be met for a valid test:

Control	Target		
	N gene (FAM)	RdRp gene (TexasRed)	RPL35A (JOE)
Positive Template Control (PTC)	+	+	+
Negative Template Control (NTC)	-	-	-

D. Data Interpretation

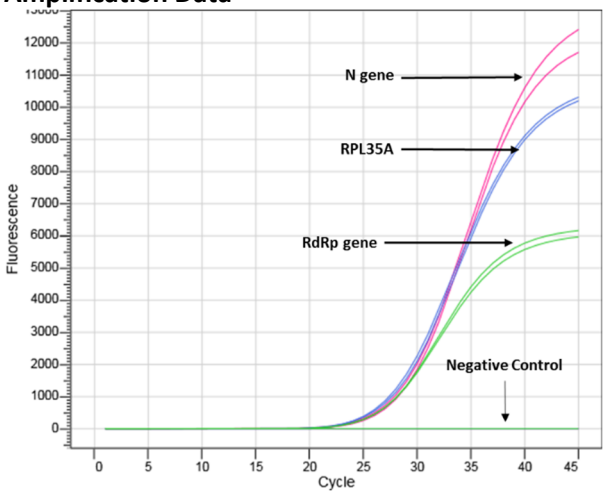
Targets			Assay Interpretation
N gene (FAM)	RdRp gene (TexasRed)	RPL35A (JOE)	
+	-	(+) or (-)	SARS-CoV-2 Positive
-	+	(+) or (-)	SARS-CoV-2 Positive
+	+	(+) or (-)	SARS-CoV-2 Positive
-	-	+	SARS-CoV-2 Negative
-	-	-	Invalid test Repeat extraction or obtain a new specimen

Ct value	Result
≤ 38	Detected (+)
> 38 or N/A	Not detected (-)

Note

- Positive results are indicative of the presence of SARS-CoV-2 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 SARS-CoV-2 infection and should not be used as the sole basis for patient management.

Amplification Data



Sr. No	Target	Ct value	
		PTC	NTC
1	N gene	29.92	--
2	RPL35A	28.28	--
3	RdRp gene	27.45	--

Note: Image representing probe based Real-Time amplification of N gene, RdRp gene and RPL35A (Ct values provided in table are for representation).

Performance Evaluation

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® COVID-19 Triplex Probe PCR Kit was conducted using clinical specimen on InstaQ96® Real Time PCR system, Bio-Rad CFX96™ C1000 Real Time PCR system and Applied Biosystems QuantStudio 5 Real-Time PCR System. The detectable limit of the Hi-PCR® COVID-19 Triplex Probe PCR Kit on both instruments was determined to be < 10 copies/reaction.

Inclusivity - Analytical Sensitivity

In-silico analysis for the assessment of inclusivity for the Hi-PCR® COVID-19 Triplex Probe PCR Kit was conducted by mapping the primers and probes against all the available SARS-CoV-2 sequences in GenBank. The Hi-PCR® COVID-19 Triplex Probe PCR Kit targets 100% of the known SARS-CoV-2 strains.

Cross-reactivity - Analytical Specificity

In-silico analysis was performed using NCBI nucleotide and Primer BLAST. The primers for RdRp and N gene were analyzed against all organisms recommended by the US FDA (data not shown because of large data set).

Wet testing analysis was performed against the following pathogens recommended by the US FDA. No cross-reaction was observed with any strains.

Human coronavirus 229E	Seasonal influenza B (Brisbane)
Human coronavirus HKU1	Seasonal influenza B (Wisconsin)
Human coronavirus NL63	Enterovirus
Human coronavirus OC43	*Candida albicans*
Middle East respiratory syndrome coronavirus (MERS-CoV)	*Staphylococcus epidermidis*
Human parainfluenza virus 1	*Hemophilus influenzae*
Human parainfluenza virus 2	*Mycobacterium tuberculosis*
Influenza A (H1N1) pandemic 2009	*Streptococcus pneumoniae*
Seasonal influenza A (H1N1)	*Legionella pneumophila*
Seasonal influenza A (H3N2)	*Pseudomonas aeruginosa*

Evaluation

Each lot of Hi-PCR® COVID-19 Triplex Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® COVID-19 Triplex 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

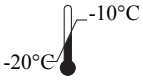
Hi-PCR® COVID-19 Triplex Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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.

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
	Do not use if package is damaged
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