Certificate number: 2019-IVD/247

Certificate of CE-Registration

This is to certify that, in accordance with the *In Vitro* Diagnostic Medical Device Directive 98/79/EC, **CEpartner4U BV** agrees to perform all duties and responsibilities as the Authorized Representative for

Himedia Laboratories PVT. LTD

23 Vadhani Industrial Estate,
LBS Marg, Mumbai - 86, MS,
India

as stipulated and demanded by the aforementioned Directive. The Dutch Competent Authorities have accepted the manufacturer's medical device registrations by CEpartner4U as listed on the product list attached to the manufacturer's Declaration of Conformity:

IVD devices were registered under number:

Group 1 :	**Dehydrated Culture Media & Supplements**	**Registration No: NL-CA002-26442**
Group 2 :	**Ready Prepared Media**	**Registration No: NL-CA002-26448**
Group 3 :	**Epidemiological Screening Kit**	**Registration No: NL-CA002-24117**
Group 4 :	**Antimicrobial Susceptibility Systems**	**Registration No: NL-CA002-26444**
Group 5 :	**Bacteriological Differentiation Aids**	**Registration No: NL-CA002-26445**
Group 6 :	**Cell Culture Media**	**Registration No: NL-CA002-26446**
Group 7 :	**Molecular Biology Products**	**Registration No: NL-CA002-26447**

see appendix

with Dutch Competent Authorities as a consequently these IVD devices were entered in EUDAMED by Dutch Competent Authorities

The manufacturer has provided CEpartner4U with all necessary documentation, together with an appropriate Declaration of Conformity that the IVD medical devices fulfil the essential requirements of Directive 98/79/EC.

Issue date: 2019-07-03

Olga Teirlinck
Consultant CEpartner4U BV

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Reason: MoldSign Signature
Location: Moldova

Supplement		Supplement		
CCM- Stem Cell Differentiation Media Supplement	TCL169	HiChondroXL™ Chondrocyte Differentiation Supplement	Low risk	28/04/2017
CCM- Stem Cell Freezing Medium	TCL107	CryoXL™ Stem Cell Freezing Medium	Low risk	28/04/2017
CCM- Stem Cell Freezing Medium	TC450U	CryoXL™DMSO, Sterile Sterile Filtered, Cell Culture Tested, Meets USP specifications	Low risk	08/12/2017
CCM-Stem Cell Differentiation Stainng Kits	CCK013	EZstain™ Adipocyte Staining Kit	Low risk	28/04/2017
CCM-Stem Cell Differentiation Stainng Kits	CCK029	EZstain™ Chondrocyte Staining Kit	Low risk	28/04/2017
CCM-Stem Cell Differentiation Stainng Kits	CCK030	EZstain™ Osteocyte Staining Kit	Low risk	28/04/2017
CCM-Balanced Salt Solutions	TL1006	Dulbecco's Phosphate Buffered Saline 1X w/o Phenol red, Calcium and Magnesium w/o Phenol red, Calcium and Magnesium	Low risk	16/12/2017
CCM-Balanced Salt Solutions	TL1098	Hanks' Balanced Salt Solution 1X w/ Sodium bicarbonate w/o Calcium, Magnesium and Phenol red w/o Calcium, Magnesium and Phenol red	Low risk	16/12/2017
CCM-Antibiotic Solutions	A033	Amphotericin B 250microgram per ml with 250 microgram per ml amphotericin B in normal saline	Low risk	16/12/2017
CCM-Antibiotic Solutions	A002	Antibiotic Antimycotic Solution 100X Liquid w/10,000 U Penicillin, 10mg Streptomycin and 25µg Amphotericin B per ml in 0.9% normal saline	Low risk	16/12/2017
CCM- Animal Cell Culture Medium Liquid	AL183	Dulbecco's Modified Eagle Medium (DMEM), Low glucose w/ 1gm Glucose per litre, Sodium bicarbonate and Sodium pyruvate w/o L-Glutamine and Phenol red	Low risk	16/12/2017

Product group	**Type/ Model / Ref number**	**Device Name**	**Risk Class**	**Date of CE compliance**
Product Group 7: Molecular Biology Products			**Registration No: NL-CA002-26447**	
MBP- DNA Isolation Kits	MB541	HiPurA™ SPP Blood DNA Isolation Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB504	HiPurA™ Blood Genomic DNA Miniprep Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB506	HiPurA™ Mammalian Genomic DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB516	HiPurA™ Blood Genomic DNA Midiprep Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB517	HiPurA™ Blood Genomic DNA Maxiprep Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB521	HiPurA™ 96 Blood Genomic DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB522	HiPurA™ Sperm Genomic DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB525	HiPurA™ Bone DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB531	HiPurA™ Buccal DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB544	HiPurA™ Stool DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB545	HiPurA™ Mycobacterium tuberculosis DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB545D	HiPurA™ Mycobacterium tuberculosis Decontamination Kit	Low risk	28/04/2017
MBP- DNA Isolation Kits	MB554	HiPurA™ Multi-Sample DNA Purification Kit	Low risk	28/04/2017
MBP-RNA Extraction Kits	MB615	HiPurA™ Viral RNA Purification Kit	Low risk	28/04/2017
MBP-RNA Extraction Kits	MB617	HiPurA™ All Blood RNA Purification Kit	Low risk	25/08/2016
MBP- DNA Isolation Kits	MB573	HiPurA™Urine DNA Purification Kit	Low risk	04/07/2018
MBP- DNA Isolation Kits	MB575	HiPurA™ Viral DNA Purification Kit	Low risk	04/07/2018
MBP- DNA Isolation Kits	MB578	HiPurA™ QuickBone DNA Purification Kit (Column Based)	Low risk	22/04/2019
MBP- DNA Isolation Kits	MB579	HiPurA™ Fast MTB (Mycobacterium tuberculosis) Genomic DNA Purification Kit	Low risk	22/04/2019
MBP- Latex Agglutination Kits	LK01	HiClostridium™ difficile Latex Test Kit	Low risk	20/12/2012
MBP- Latex Agglutination Kits	LK02	HiSalmonella™ Latex Test Kit	Low risk	20/12/2012

CEpartner4U

Certificate number: 2020-IVD/293

Certificate of CE-Registration

This is to certify that, in accordance with the *In Vitro* Diagnostic Medical Device Directive 98/79/EC, **CEpartner4U BV** agrees to perform all duties and responsibilities as the Authorized Representative for

Himedia Laboratories PVT. LTD

23 Vadhani Industrial Estate,
LBS Marg, Mumbai - 86, MS,
India

as stipulated and demanded by the aforementioned Directive. The Dutch Competent Authorities have accepted the manufacturer's medical device registrations by CEpartner4U as listed on the product list attached to the manufacturer's Declaration of Conformity:

IVD devices were registered under number:

Group 7 : Molecular Biology Products
Registration No: NL-CA002-26447

see appendix

with Dutch Competent Authorities as a consequently these IVD devices were entered in EUDAMED by Dutch Competent Authorities

The manufacturer has provided CEpartner4U with all necessary documentation, together with an appropriate Declaration of Conformity that the IVD medical devices fulfil the essential requirements of Directive 98/79/EC.

Issue date: 2020-09-09

Olga Teirlinck
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Appendix

Product Group	Device Name	Type/Model/Ref Number	Risk Class
MBP-RNA Extraction Kits	HiPurA® Viral RNA Automated Extraction Kit	MB615MA	Low
MBP-RNA Extraction Kits	HiPurA® Tissue RNA Purification Kit	MB618	Low
MBP-DNA Isolation Kits	HiPurA® DNA/RNA Purification Kit	MB583	Low
MBP-DNA Isolation Kits	HiPurA® Viral DNA/RNA Purification Kit	MB582	Low
MBP-DNA Isolation Kits	InstaNX® Blood DNA Plus Purification Kit	MBIN025	Low
MBP-DNA Isolation Kits	InstaNX® Viral DNA Plus Purification Kit	MBIN026	Low
MBP-RNA Extraction Kits	InstaNX® Tissue/Cell RNA Purification Kit	MBIN027	Low
MBP-Hematology Kits	Alkaline Paper Hemoglobin Electrophoresis Kit	MBP009	Low
MBP-PCR Kits	Hi-PCR® Generic E. coli Probe PCR Kit	MBPCR153	Low
MBP-PCR Kits	Hi-PCR® Total Coliform Probe PCR Kit	MBPCR200	Low
MBP-PCR Kits	Hi-PCR® Dengue Serotyping Probe PCR Kit	MBPCR137	Low
MBP-PCR Kits	Hi-PCR® Generic E. coli Probe PCR Kit	MBPCR238	Low
MBP-PCR Kits	Hi-PCR® E.coli O157:H7 Probe PCR Kit	MBPCR154	Low
MBP-PCR Kits	Hi-PCR® Vibrio cholerae Probe PCR Kit	MBPCR156	Low
MBP-PCR Kits	Hi-PCR® Enterovirus Probe PCR Kit	MBPCR201	Low
MBP-PCR Kits	Hi-PCR® Salmonella Quantification Probe PCR Kit	MBPCR196	Low
MBP-PCR Kits	Hi-PCR® E.coli O157:H7 Quantification Probe PCR Kit	MBPCR198	Low
MBP-PCR Kits	Hi-PCR® ColistinResistance Probe PCR Kit	MBPCR209	Low
MBP-RNA Extraction Kits	HiPurA® Viral RNA Purification Kit (Magnetic Bead Based)	MB615M	Low
MBP-RNA Extraction Kits	Pre-filled Plates for Insta NX® Mag32	MB615MPF-32	Low
MBP-RNA Extraction Kits	Pre-filled Plates for Insta NX® Mag96	MB615MPF-96	Low
MBP PCR Kits	Hi-PCR® Coronavirus (COVID-19) Multiplex Probe PCR Kit	MBPCR243	Low

MBPCR243 Hi-PCR® Coronavirus (COVID-19) Multiplex 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® Coronavirus (COVID-19) Multiplex Probe PCR Kit is for *in-vitro* use only.

Intended Use

Hi-PCR® Coronavirus (COVID-19) 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 SARS-CoV-2 in clinical samples.

Product Description

Hi-PCR® Coronavirus (COVID-19) Multiplex Probe PCR Kit includes primer-probe sets specific to Envelope Protein (E) gene, 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 ribonuclease P RNA component H1 (RPPH1) 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.

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

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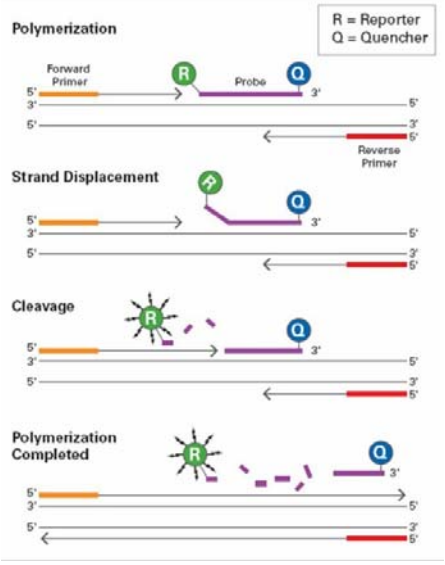
The information contained herein is believed to be accurate and complete. However no warranty or guarantee whatsoever is made or is to be implied with respect to such information or with respect to any product, method or apparatus referred to herein

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 Cy5 channels respectively with detection of Envelope (E) gene specific for Sarbecovirus in TexasRed channel. The RPPH1 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: Bronchoalveolar lavage, tracheal aspirate, sputum, nasopharyngeal swab, oropharyngeal swab, nasopharyngeal wash/aspirate or nasal aspirate.

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. 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)*	
		10R	50R
RT Buffer	DS0221	0.055 mL	0.3 mL
10X Solution H	DS0222	0.03 mL	0.15 mL
M-MuLV Reverse Transcriptase	DS0220	0.015 mL	0.075 mL
nCoV Multiplex Primer-Probe Mix	DS0988	0.05 mL	0.25 mL
nCoV Multiplex Positive Control	DS0997	0.06 mL	0.3 mL
Water	DS0440	0.5 mL	2.5 mL

* For a 25 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
TabSpin™ Microcentrifuge	LA1089/LA1090
Automated nucleic acid extraction system and materials	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Viral RNA Purification Kit	MBIN013
Kits and Reagents	
HiPurA® Viral RNA Purification Kit (CDSCO approved)	MB615
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
8-strip tubes & optically clear flat caps for PCR	PR17
PCR Tubes, 0.2 mL; PCR Plates	PW1255/ PR2/PR3/PR19
Optical Sealing film	PR18
RNase Kil™	ML162

Kit Compatibility with Real-Time PCR systems:

Hi-PCR® Coronavirus (COVID-19) Multiplex 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® 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™ 5 Real‑Time PCR Instrument (Applied Biosystems)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- Corbett Rotor-Gene® 6000 (QIAGEN)
- LightCycler® 480 Instrument II (Roche)

Note: Ensure that the Real-Time PCR system is calibrated for FAM dye, JOE/HEX dye, Texas Red dye, and CY5 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/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 (µL) to be added for 1X (for a 25 µL reaction)**
RT Buffer	5
10X Solution H	2.5
M-MuLV Reverse Transcriptase	1
nCoV Multiplex Primer-Probe Mix	4
Test – Extracted Sample RNA	5
Positive Control/Test Sample/Negative Control	-
Water	7.5
Total volume	25

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 20 µL of master mixture into the 0.1/0.2 mL PCR reaction plate/strips, compatible to the instrument to be used, add 5 µL of the negative control.
5. In the "Nucleic acid handling" area, add nCoV Multiplex 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 3 minutes	
3.	Denaturation	:	95°C for 15 seconds	No. of cycles: 40
4.	Annealing	:	58°C for 30 seconds (Plate Read)	
	Channel	:	FAM/JOE[#]/TexasRed/Cy5	
5.	Hold	:	4°C for ∞	

[#]for instruments not calibrated for JOE, HEX can be used

Data Analysis

The following conditions should be met for a valid test:

Control	Target			
	N gene (FAM)	E gene (TexasRed)	RdRp gene (Cy5)	RPPH1 (JOE)
Positive Template Control (PTC)	+	+	+	+
Negative Template Control (NTC)	-	-	-	-

Data Interpretation

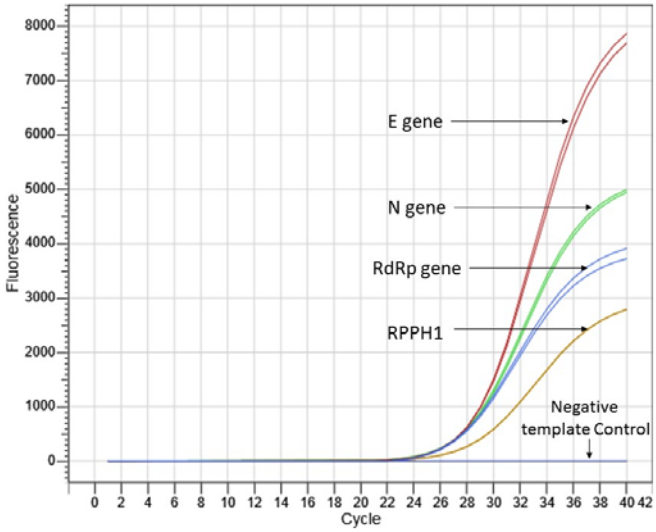
Targets				Assay Interpretation
N gene (FAM)	E gene (TexasRed)	RdRp gene (Cy5)	RPPH1 (JOE)	
+	+	+	(+) or (-)	SARS-CoV-2 Positive
+	+	-	(+) or (-)	SARS-CoV-2 Positive
-	+	+	(+) or (-)	SARS-CoV-2 Positive
+	-	+	(+) or (-)	SARS-CoV-2 Positive
+	-	-	(+) or (-)	Presumptive Positive *Test again, and if same result observed: SARS-CoV-2 Positive*
-	-	+	(+) or (-)	Presumptive Positive *Test again, and if same result observed: SARS-CoV-2 Positive*
-	+	-	(+) or (-)	Presumptive Positive Test again, with higher volume of template RNA (up to 10µl) *If positive for other targets: SARS-CoV-2 Positive* *If negative for other targets: Test using a fresh specimen*
-	-	-	+	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	C_t value	
		PC	NTC
1.	E gene	27.55	-
2.	N gene	27.6	-
3.	RdRp gene	27.65	-
4.	RPPH1	29.6	-

Note: Image representing probe based Real-Time amplification of E gene, N gene RdRp gene and RPPH1 gene (Ct values provided in table are for representation).

Performance Evaluation

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® Coronavirus (COVID-19) Multiplex Probe PCR Kit was conducted using clinical specimens on InstaQ96® Real Time PCR system and Bio-Rad CFX96™ C1000 Real Time PCR system. The detectable limit of the Hi-PCR® Coronavirus (COVID-19) Multiplex 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® Coronavirus (COVID-19) Probe PCR Kit was conducted by mapping the primers and probes against all the available SARS-CoV-2 sequences in GenBank. The Hi-PCR® Coronavirus (COVID-19) Multiplex 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 E, 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® Coronavirus (COVID-19) Multiplex Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® Coronavirus (COVID-19) 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® Coronavirus (COVID-19) 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

IVD	In vitro diagnostic medical device
CE	CE Marking
-10°C -20°C	Storage temperature
	Do not use if package is damaged
	Reg. Off : 23, Vadhani Ind Est., LBS Marg, Mumbai-400086, India. Works : B-4-5-6 / MIDC, Palkhed, Dindori, Nashik- 422202 Maharashtra, India www.himedialabs.com
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Vienna, 08 April 2019

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Registration No.: 00275/0

Date of initial issue: 21 November 2017

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MB615 HiPurA® Viral RNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB615		
		20 Preps	50 preps	250 preps
DS0037	RNA Lysis Solution (HRL)	16 ml	40 ml	200 ml
DS0012	Wash Solution Concentrate (WS)	6 ml	15 ml	75 ml
DS0042	Elution Solution (RNase- Free Water)	2.4 ml	6 ml	30 ml
DS0192	Carrier RNA	0.28 mg	0.7 mg	3.5 mg
DBCA03	HiElute Miniprep Spin Column (Capped) [in Uncapped Collection Tube]	20 nos	50 nos	250 nos
PW146	Micro Centrifugal Tube-B (1.5ml)	20 nos	50 nos	250 nos
PW1139	Collection Tube, Polypropylene (2.0 ml)	20 nos	50 nos	250 nos

Intended Use

Recommended for isolation of Viral RNA from various samples like fresh and frozen plasma, serum, nasopharyngeal swab, oropharyngeal swab, sputum, BAL in Viral Transport Medium and other body fluids.

Introduction

HiPurA® Viral RNA Purification Kit provide the fastest and easiest way to purify viral RNA for reliable use in amplification technologies. Viral RNA can be purified from plasma (treated with anticoagulant EDTA), serum, other body fluids, and infected tissues. Samples may be fresh or frozen, but if frozen, should not be thawed more than once. Repeated freeze–thawing of plasma samples will lead to reduced viral titers and should be avoided for optimal sensitivity. HiPurA® Viral RNA Purification Kit can be used for isolation of viral RNA from a wide variety of viruses, but performance may vary depending on virus type.

HiPurA® Viral RNA Purification Kit

This kit carries out efficient extraction of viral RNA from wide range of viral strains like Influenza, Dengue, Chikungunya and viral pathogen of animals. Sample is first lysed under the highly denaturing conditions provided by Buffer HRL to inactivate RNases and to ensure isolation of intact viral RNA. When Carrier RNA is added to Elution Solution (RNase-free Water), it improves the binding of viral RNA to the HiElute Miniprep Spin Column especially in the case of low-titer samples, and limits possible degradation

Elution

The yield of RNA depends on the sample type and the number of cells in the sample. A single elution with 60-80µl of Elution Solution will provide sufficient RNA to carry out multiple amplification reactions. **NOTE:** For more concentrated RNA lower elution volume (30-40 µl) can be used. Larger elution volumes (up to 100 µl) can also be used but may result in dilution of viral RNA sample.

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HiElute Miniprep Spin Column (Capped) (DBCA03)

HiElute Miniprep Spin Column (Capped) is based on the advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced gel membrane and the speed plus versatility of spin column technology to yield high quantity of RNA. The use of spin column facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. This column eliminates the need for alcohol precipitation, expensive resins, and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional RNA isolation techniques. RNA binds specifically to the advanced silica-gel membrane while contaminants pass through. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in the buffer provided with the kit.

Storage

HiPurA® Viral RNA Purification Kit can be stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance. Store the DS0192- Carrier RNA in -20°C temperature on receipt. We recommend storing the reconstituted Carrier RNA at -20°C in aliquots to avoid repeated freeze and thaw.

Materials needed but not provided

- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- RNase – free pipette tips (aerosol barrier recommended)
- Ethanol (96 – 100%)

Precautions to be taken while handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non- disposable vessels and solutions while working with RNA.

1. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.
2. Use sterile, disposable plasticware and autoclavable pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipments.
3. Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1M NaOH, 1mM EDTA followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.
4. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240ºC for four or more hours before use. Alternatively, glassware can be treated with DEPC (Diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight at 37°C, and then autoclave or heat to 100°C for 15 min to eliminate residual DEPC.
5. Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
6. Solutions (water and other solutions) should be treated with 0.1% DEPC

General Preparation Instructions

1. **Thoroughly mix reagents**
   Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.

2. Ensure that clean & dry Nuclease-free tubes and tips are used for the procedure.

3. **Reconstitute Carrier RNA**

Number of Preps	Carrier RNA	Elution Buffer (RNase free water)
20	0.28 mg	280 µl
50	0.7 mg	700 µl
250	3.5 mg	3.5 ml

   Dissolve Carrier RNA thoroughly by pipetting. We recommend storing the reconstituted Carrier RNA at -20°C in aliquots to avoid repeated freeze and thaw.

4. **Preparation of Carrier RNA –Lysis Solution (HRL)**

Number of Preps	Volume of Carrier RNA	Volume of Lysis Solution (HRL)
20	112 µl	11.2 ml
50	280 µl	28 ml
250	1.4 ml	140 ml

   NOTE: Concentration of Carrier RNA to be used is 10µg/ml

   Calculate the volume of Carrier RNA –Lysis Solution (HRL) as follows:

   a X 0.56 ml = **b** ml

   b ml X 10 µl/ml = **c** µl

where, **a** = number of sample to be processed
b = volume of Lysis Solution (HRL) to be added for '**a**' number of samples
c = volume of Carrier RNA to be added to Lysis Buffer (HRL)

eg: for 2 number of samples, add 1.12 ml of Lysis Solution (HRL) and 11.2 µl of Carrier RNA

Number of Preps	Lysis Solution (HRL) ml	Reconstituted Carrier RNA µl	Number of Preps	Lysis Solution (HRL) ml	Reconstituted Carrier RNA µl
1	0.56	5.6	13	7.28	72.8
2	1.12	11.2	14	7.84	78.4
3	1.68	16.8	15	8.40	84.0
4	2.24	22.4	16	8.96	89.6
5	2.80	28.0	17	9.52	95.2
6	3.36	33.6	18	10.08	100.8
7	3.92	39.2	19	10.64	106.4
8	4.48	44.8	20	11.20	112.0
9	5.04	50.4	21	11.76	117.6
10	5.60	56	22	12.32	123.2
11	6.16	61.6	23	12.88	128.8
12	6.72	67.2	24	13.44	134.4

5. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
20	6 ml	18 ml
50	15 ml	45 ml
250	75 ml	225 ml

Specimen Handling and Collection

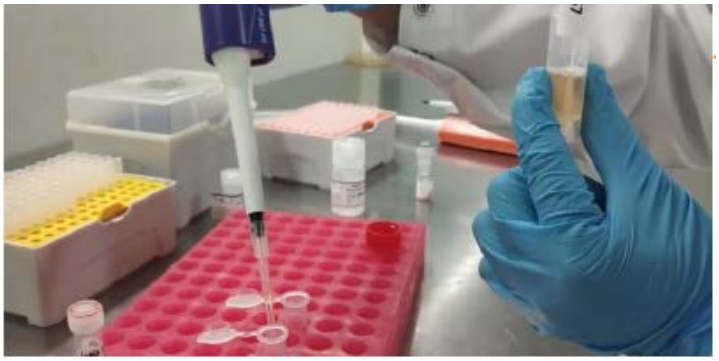
Collect plasma, serum or other body fluids in a sterile container. Thaw the samples on ice before use. Repeated freeze- thaw of samples should be avoided.

Type of Specimens: Clinical samples (Serum, plasma, swabs in viral transport medium and other body fluids)

Procedure

1. Add 140 µl of sample like serum, plasma or body fluid, nasopharyngeal swab, oropharyngeal swab, sputum, BAL, samples collected in Viral Transport medium to Collection Tube, Polypropylene (2.0 ml) (PW1139).

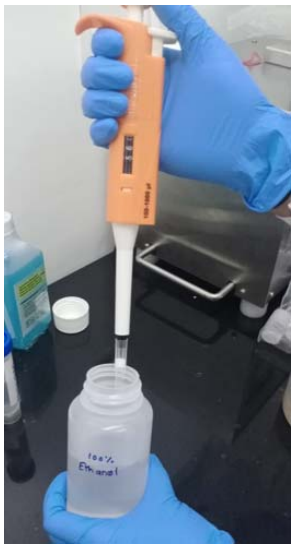
   NOTE: The procedure is optimized for use with 140 µl samples but upto 300 µl sample can be used. During work with smaller sample volume (~100 µl) it should be made up to 140 µl with PBS (Phosphate Buffered Saline) before processing. If the initial sample volume is increased, application of the lysed sample to the HiElute Miniprep Spin Column will require multiple loading steps.



2. Add 560 µl of Carrier RNA-Lysis Solution (HRL) to the sample. (**Refer to General Preparation Instructions)**. Mix by pulse vortexing for 15 seconds.

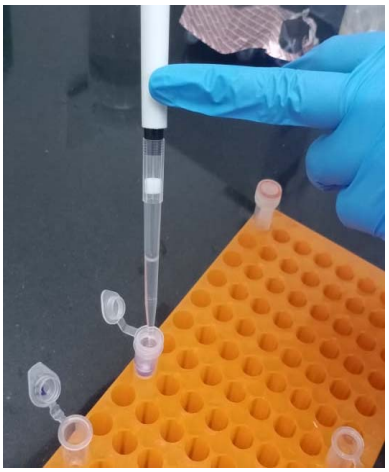
3. Incubate for 10 minutes at room temperature (15-25°C).
4. Centrifuge the samples for 10 seconds to remove any droplets formed inside the cap of collection tubes.

5. **Binding**
   Add 560 µl of ethanol (96-100%) to the sample, mix well by gentle pipetting.



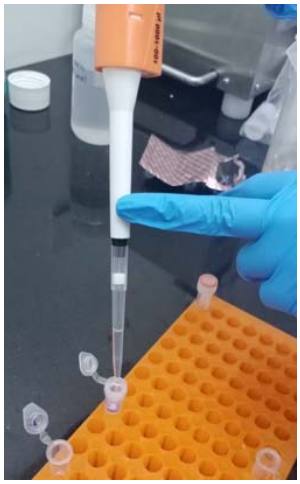
6. Centrifuge the samples for 10 seconds to remove any droplets formed inside the cap of collection tubes.

7. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Transfer the lysate obtained in step 6 onto the HiElute Miniprep Spin Column. Centrifuge at 8,000 rpm for 1 minute.



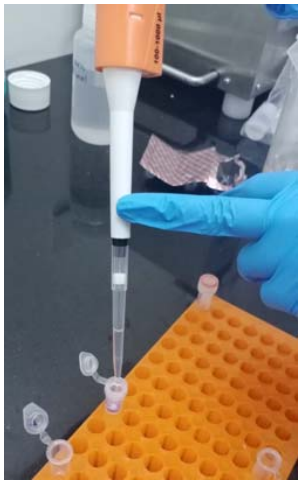

8. Discard the flow-through after the spin. Repeat step 7 with the remaining sample. Reuse the collection tube.

9. **First Wash**
   (Prepare Wash Solution as indicated in General Preparation Instructions)
   Add 500 µl of diluted Wash Solution (WS) (DS0012). Centrifuge at 8,000 rpm for 1 minute.

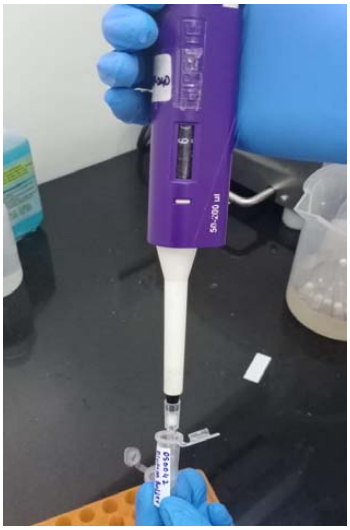

10. Discard the flow-through. Reuse the collection tube.

11. **Second Wash**
    Add another 500 µl of diluted Wash Solution (WS) (DS0012) onto the column. Close the tube gently and centrifuge for 3 minutes at 14,000 rpm to wash the column.



12. Discard the flow-through. Reuse the collection tube. Centrifuge for 1 minute at 14,000 rpm to dry the membrane.

13. Transfer the HiElute Miniprep Spin column (Capped) (DBCA03) to a Micro Centrifugal Tube 1.5ml (PW146). Pipet 60-80 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Incubate for 1 minutes at room temperature (15-25°C).


14. Close the tube gently and centrifuge for 1 minute at 8,000 rpm. The eluate in the Micro Centrifugal Tube 1.5ml (PW146) contains pure RNA.
NOTE: Place the Micro Centrifugal Tube 1.5ml (PW146) at alternate position in the rotor of the centrifuge machine. The cap of the Micro Centrifugal Tube 1.5ml (PW146) might break if kept side by side in the rotor of centrifuge machine.

Storage of the eluate with purified RNA: The eluate contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

Warning

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.

Performance and Evaluation

Yield of viral RNA isolated from biological samples is usually very less (approx. <1 µg). As a result it is difficult to measure the yield spectrophotometrically. Another point to keep in mind that the carrier RNA will account for for most of the RNA present. The yield and efficiency of purification is determined by performing Quantitative RT-PCR. All the QC passed batches have atleast 90% recovery of the viral RNA.

Quality Control

Each lot of HiMedia's HiPurA® Viral RNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

References

1. Sambrook, J.,*et al*. Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989)
2. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A practical guide (Academic Press, San Diego, CA, 1993).

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Clogged HiElute Miniprep Spin Column (Capped)	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
		Centrifugation temperature is too low	The centrifugation temperature should be 20 – 25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol containing lysate to 37°C before transferring it to the column.
2.	Low RNA Yield	Too much of starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
		RNA still bound to HiElute Miniprep Spin Column	Repeat RNA elution, but incubate the column for 10 minutes at room temperature with Elution solution (RNase free water) before centrifuging.

		Ethanol carryover	During the second wash with Wash Solution (WS) be sure to centrifuge at ≥8000 x g (≥10, 000 rpm) for 2 minutes to dry the column. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow through otherwise carryover of ethanol will occur. To eliminate any chance of possible ethanol, centrifuge the column for another step minute at full speed.
		No DNase treatment	Follow the optional on-column DNase digestion
5.	RNA does not perform well in downstream experiments	Ethanol carryover	During the second Wash using Wash Solution (WS), be sure to dry the HiElute Miniprep Spin Column membrane by centrifugation at ≥8000 x g (≥10,000 rpm) for 2 minutes to dry the membrane. Following the centrifugation, remove the HiElute Miniprep Spin Column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

Safety Information

The HiPurA® Viral RNA Purification Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

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 to mb@himedialabs.com.

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MBPCR243 Hi-PCR® Coronavirus (COVID-19) Multiplex 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® Coronavirus (COVID-19) Multiplex Probe PCR Kit is for *in-vitro* use only.

Intended Use

Hi-PCR® Coronavirus (COVID-19) 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 SARS-CoV-2 in clinical samples.

Product Description

Hi-PCR® Coronavirus (COVID-19) Multiplex Probe PCR Kit includes primer-probe sets specific to Envelope Protein (E) gene, 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 ribonuclease P RNA component H1 (RPPH1) 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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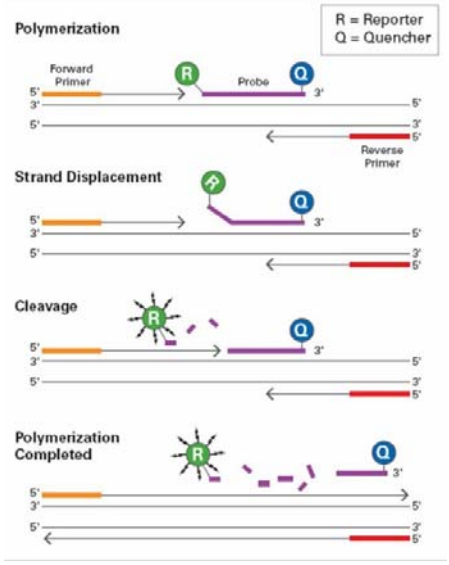
Principle

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 Cy5 channels respectively with detection of Envelope (E) gene specific for Sarbecovirus in TexasRed channel. The RPPH1 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
RT Buffer	DS0221	0.3 mL	0.6 mL
10X Solution H	DS0222	0.15 mL	0.3 mL
M-MuLV Reverse Transcriptase	DS0220	0.075 mL	0.15 mL
nCoV Multiplex Primer-Probe Mix	DS0988	0.25 mL	0.5 mL
nCoV Multiplex Positive Control	DS0997	0.3 mL	0.6 mL
Water	DS0440	2.5 mL	2 X 2.5 mL

* For a 25 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
TabSpin™ Microcentrifuge	LA1089/LA1090
Automated nucleic acid extraction system and materials	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Viral RNA Purification Kit	MBIN013
Kits and Reagents	
HiPurA® Viral RNA Purification Kit (CDSCO approved)	MB615
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
8-strip tubes & optically clear flat caps for PCR	PR17
PCR Tubes, 0.2 mL; PCR Plates	PW1255/ PR2/PR3/PR19
Optical Sealing film	PR18
RNase Kil™	ML162

Kit Compatibility with Real-Time PCR systems:

Hi-PCR® Coronavirus (COVID-19) Multiplex 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® 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™ 5 Real‑Time PCR Instrument (Applied Biosystems)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- Corbett Rotor-Gene® 6000 (QIAGEN)
- LightCycler® 480 Instrument II (Roche)

Note: Ensure that the Real-Time PCR system is calibrated for FAM dye, JOE/HEX dye, Texas Red dye, and CY5 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/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 (µL) to be added for 1X (for a 25 µL reaction)**
RT Buffer	5
10X Solution H	2.5
M-MuLV Reverse Transcriptase	1
nCoV Multiplex Primer-Probe Mix	4
Test – Extracted Sample RNA	5
Positive Control/Test Sample/Negative Control	-
Water	7.5
Total volume	25

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 20 µL of master mixture into the 0.1/0.2 mL PCR reaction plate/strips, compatible to the instrument to be used, add 5 µL of the negative control.
5. In the "Nucleic acid handling" area, add nCoV Multiplex 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 3 minutes	
3.	Denaturation	:	95°C for 15 seconds	No. of cycles: 40
4.	Annealing	:	58°C for 30 seconds (Plate Read)	
	Channel	:	FAM/JOE[#]/TexasRed/Cy5	
5.	Hold	:	4°C for ∞	

[#]for instruments not calibrated for JOE, HEX can be used

Data Analysis

The following conditions should be met for a valid test:

Control	Target			
	N gene (FAM)	E gene (TexasRed)	RdRp gene (Cy5)	RPPH1 (JOE)
Positive Template Control (PTC)	+	+	+	+
Negative Template Control (NTC)	-	-	-	-

Data Interpretation

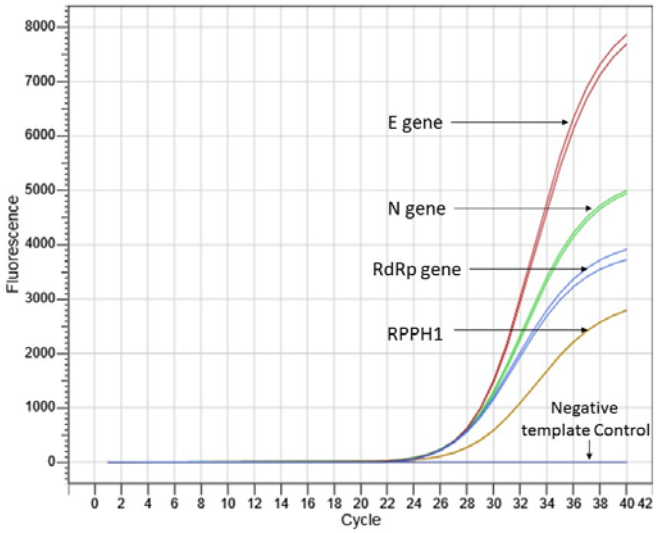
Targets				Assay Interpretation
N gene (FAM)	E gene (TexasRed)	RdRp gene (Cy5)	RPPH1 (JOE)	
+	+	+	(+) or (-)	SARS-CoV-2 Positive
+	+	-	(+) or (-)	SARS-CoV-2 Positive
-	+	+	(+) or (-)	SARS-CoV-2 Positive
+	-	+	(+) or (-)	SARS-CoV-2 Positive
+	-	-	(+) or (-)	Presumptive Positive *Test again, and if same result observed: SARS-CoV-2 Positive*
-	-	+	(+) or (-)	Presumptive Positive *Test again, and if same result observed: SARS-CoV-2 Positive*
-	+	-	(+) or (-)	Presumptive Positive Test again, with higher volume of template RNA (up to 10µl) *If positive for other targets: SARS-CoV-2 Positive* *If negative for other targets: Test using a fresh specimen*
-	-	-	+	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	C_t value	
		PC	NTC
1.	E gene	27.55	-
2.	N gene	27.6	-
3.	RdRp gene	27.65	-
4.	RPPH1	29.6	-

Note: Image representing probe based Real-Time amplification of E gene, N gene RdRp gene and RPPH1 gene (Ct values provided in table are for representation).

Performance Evaluation

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® Coronavirus (COVID-19) Multiplex Probe PCR Kit was conducted using clinical specimens on InstaQ96® Real Time PCR system and Bio-Rad CFX96™ C1000 Real Time PCR system. The detectable limit of the Hi-PCR® Coronavirus (COVID-19) Multiplex 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® Coronavirus (COVID-19) Probe PCR Kit was conducted by mapping the primers and probes against all the available SARS-CoV-2 sequences in GenBank. The Hi-PCR® Coronavirus (COVID-19) Multiplex 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 E, 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® Coronavirus (COVID-19) Multiplex Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® Coronavirus (COVID-19) 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® Coronavirus (COVID-19) 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

IVD	In vitro diagnostic medical device
CE	CE Marking
-10°C -20°C	Storage temperature
	Do not use if package is damaged
	Reg. Off : 23, Vadhani Ind Est., LBS Marg, Mumbai-400086, India. Works : B-4-5-6 / MIDC, Palkhed, Dindori, Nashik- 422202 Maharashtra, India www.himedialabs.com
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

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Disclaimer :

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