# MBPCR270 Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit

## Instructions For Use

### Description

The coronavirus disease 2019 (COVID-19) pandemic is an ongoing global health crisis caused by a newly discovered coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The COVID-19 pandemic began in December 2019 in Wuhan City, China. Clinical presentation of COVID-19 ranged from asymptomatic cases and mild illness to critical conditions featuring acute respiratory distress syndrome. Influenza virus (Flu) and respiratory syncytial virus (RSV) are common respiratory pathogens that cause seasonal epidemics. Influenza infection tends to be less severe, typically resulting in uncomplicated upper respiratory tract illness, but, in rare cases, influenza infection can induce a complicated disease with severe viral pneumonia. RSV is recognized as the most common cause of bronchiolitis and pneumonia in children younger than 1 year. RSV infection can produce common cold-like symptoms but may be severe in infants and young children. The clinical presentations of SARS-CoV-2, influenza, and RSV infections have overlapping symptoms, thus differential diagnosis of these diseases is challenging. Rapid diagnosis is needed in elderly patients because SARS-CoV-2, influenza, and RSV infections result in substantial morbidity and mortality specifically in these patients. In the COVID-19 pandemic era, clinicians may encounter a co-infection of SARS-CoV-2 with influenza or RSV. Therefore, multiplex detection of SARS-CoV-2, Influenza, and RSV will be very essential. HiMedia has developed a Multiplex Reverse Transcriptase Real-Time PCR kit which enables the clinicians and public health laboratories to quickly diagnose and differentiate SARS-CoV-2, Influenza and RSV infections infection in a two-tube reaction.

**NOTE**: Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit is for *in-vitro* use only.

### Intended Use

Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit is a TaqMan based chemistry. It 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, influenza and RSV infections in clinical samples.

### Product Description

Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit includes two sets of primer-probe mix which specifically detect conserved regions of six different pathogens. These include N-gene for SARS-CoV-2 virus, M-gene for Influenza A virus, HA-gene for swine flu H1N1/ H1N1 pdm09 virus, HA-gene for H3N2, NS-gene for Influenza B and NS-gene for RSV A & B virus. In addition, both the tubes contain human RPL35A ribosomal protein L35a as endogenous internal control (IC) gene to assess RNA extraction efficiency and to ensure successful PCR reaction. The kit also provides 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.



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

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**Negative Control**

A Negative control is needed to ensure that the reagents, equipment, and environment used in the assay are not contaminated with target 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.

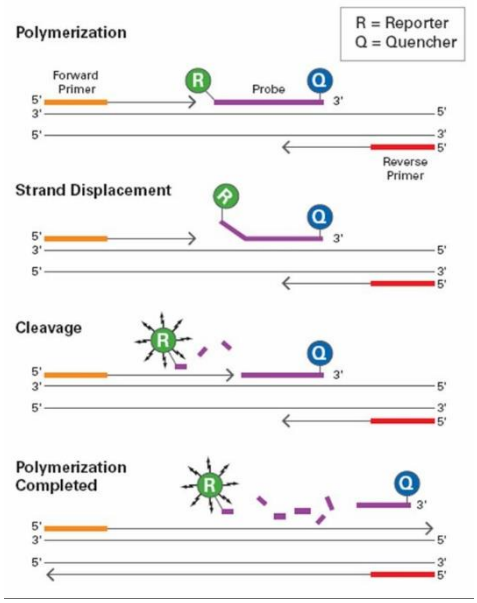
**Internal Control**

This is a control sequence that should amplify in all clinical samples in both the tubes 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 (TaqMan 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 SARS-CoV-2 virus, influenza A virus (swine flu H1N1 & H3N2), influenza B virus and RSV specific RNA targets in a two-tube reaction.

**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 within 2 hours
- Highly sensitive and specific for detection of SARS-CoV-2, influenza and RSV infections
- 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 -80°C 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 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 a maximum of 10 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 the pack.

**Kit Contents:** The provided PCR kit contains:

| Components | Product Codes | Reagents provided for (reactions)* | | |
|---|---|---|---|---|
| | | 25R | 50R | 100R |
| CoV FLU RSV Master Mix | DS1394 | 0.729 mL | 1.431 mL | 2.781 mL |
| CoV FLU RSV Primer-Probe Mix 1 | DS1378 | 0.0405 mL | 0.081 mL | 0.159 mL |
| CoV FLU RSV Primer-Probe Mix 2 | DS1386 | 0.0405 mL | 0.081 mL | 0.159 mL |
| CoV FLU RSV Positive Control 1 | DS1392 | 0.054 mL | 0.108 mL | 0.212 mL |
| CoV FLU RSV Positive Control 2 | DS1393 | 0.054 mL | 0.108 mL | 0.212 mL |
| Water | DS0440 | 0.054 mL | 0.108 mL | 0.212 mL |

**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 |
| Q4Pet Autoclavable Micropipette Capacity: 100-1000µL/10-100µL | MBLA008/MBLA011 |
| Q4Pet Autoclavable Micropipette Capacity: 0.5-10µL/20-200µL | MBLA009/ MBLA012 |
| 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/ Micro Centrifuge Tube-C | PW146/ 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/ Optical Sealing film/ RNase Kil™ | PR21/ PR18/ ML162 |

**Kit Compatibility with Real-Time PCR systems:**

Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit contains fluorophores compatible to:

| Real-Time PCR system | Company | Dye 1 (H1N1/ Inf B) | Dye 2 (SARS-CoV-2/ H3N2) | Dye 3 (IC) | Dye 4 (Inf A/ RSV) |
|---|---|---|---|---|---|
| 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 CFX Opus 96/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**

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 to in the 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.
- Do not vortex the reagents, instead mix the contents of the vial by gently pipetting.

**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 follows

**Note:** Two separate tubes must be run for a single sample as follows.

| Components | Tube – 1 | Tube – 2 |
|---|---|---|
| CoV FLU RSV Master Mix | 13.5 µL | 13.5 µL |
| CoV FLU RSV Primer-Probe Mix – 1 | 1.5 µL | --- |
| CoV FLU RSV Primer-Probe Mix – 2 | --- | 1.5 µL |
| **Total volume of Master Mix** | **15 µL** | **15 µL** |
| Template | 10.0 µL | 10.0 µL |
| **Total reaction volume** | **25 µL** | **25 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 15.0 µL of master mix into the 0.1/0.2 mL PCR reaction plate/strips, compatible to the instrument to be used.
5. Add 10 µL of nuclease free water to the negative control tube in the master mix preparation area.
6. In the "Nucleic acid handling" area, add 10.0 µL of CoV FLU RSV Positive Control 1 and CoV FLU RSV Positive Control 2 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 the Real-time PCR machine and set the PCR program.

**A. Recommended PCR program**

| Step | Temperature | Time | Sampling | Cycles |
|---|---|---|---|---|
| 1 | 55°C | 20 minutes | --- | 1 |
| 2 | 95°C | 02 minutes | --- | 1 |
| 3 | 95°C | 15 seconds | --- | 45 |
| 4 | 55°C | 45 seconds | Yes | |
| 5 | 72°C | 15 seconds | --- | |

**B. Selection of channel**

| Tube | Target | Dye | Quencher |
|---|---|---|---|
| Tube - 1 | H1N1 | FAM | None |
| | SARS-CoV-2 | JOE/VIC/HEX | None |
| | IC | ROX/Texas Red | None |
| | Inf A | Cy5 | None |
| Tube - 2 | Inf B | FAM | None |
| | H3N2 | JOE/VIC/HEX | None |
| | IC | ROX/Texas Red | None |
| | RSV | Cy5 | None |

**Passive Reference Dye:** Select **"**None"

**C. PCR Data Analysis**

The following conditions should be met for a valid test:

| Controls | Tube - 1 | | | | Tube - 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | H1N1 (FAM) | SARS-CoV-2 (JOE) | IC (ROX) | Inf A (Cy5) | Inf B (FAM) | H3N2 (JOE) | IC (ROX) | RSV (Cy5) |
| **Positive Template Control (PTC)** | + | + | + | + | + | + | + | + |
| **Negative Template Control (NTC)** | - | - | - | - | - | - | - | - |

## D. Data Interpretation

**FOR SINGLE VIRUS INFECTIONS**

| Targets in Tube -1 | | | | Targets in Tube -2 | | | | Assay Interpretation |
|---|---|---|---|---|---|---|---|---|
| H1N1 (FAM) | SARS-CoV-2 (JOE) | IC (ROX) | Inf A (Cy5) | Inf B (FAM) | H3N2 (JOE) | IC (ROX) | RSV (Cy5) | |
| - | + | + | - | - | - | + | - | Positive for SARS-CoV-2 virus |
| - | - | + | + | - | - | + | - | Positive for Influenza A* virus |
| + | - | + | + | - | - | + | - | Positive for Influenza A (H1N1) virus |
| - | - | + | + | - | + | + | - | Positive for Influenza A (H3N2) virus |
| - | - | + | - | + | - | + | - | Positive for Influenza B virus |
| - | - | + | - | - | - | + | + | Positive for RSV |
| - | - | + | - | - | - | + | - | Negative for SARS-CoV-2, Influenza & RSV |
| - | - | - | - | - | - | - | - | Invalid test. Repeat extraction or obtain a new specimen |

**FOR MULTIPLE VIRAL CO-INFECTIONS**

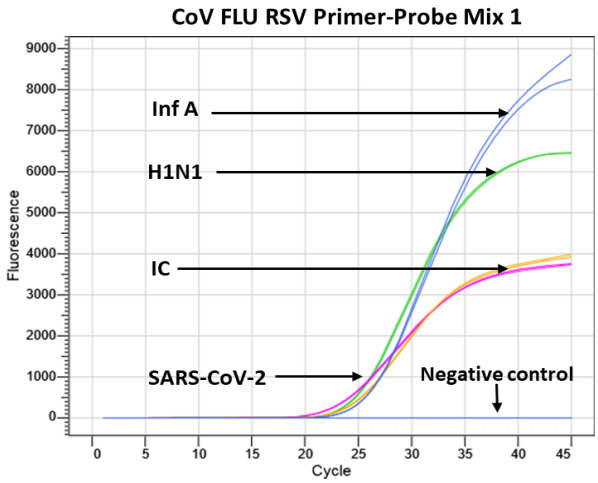
| Targets in Tube -1 | | | | Targets in Tube -2 | | | | Assay Interpretation |
|---|---|---|---|---|---|---|---|---|
| H1N1 (FAM) | SARS-CoV-2 (JOE) | IC (ROX) | Inf A (Cy5) | Inf B (FAM) | H3N2 (JOE) | IC (ROX) | RSV (Cy5) | |
| - | + | + | + | - | - | + | - | SARS-CoV-2 & Influenza A* coinfection |
| + | + | + | + | - | - | + | - | SARS-CoV-2 & Influenza A (H1N1) virus coinfection |
| - | + | + | + | - | + | + | - | SARS-CoV-2 & Influenza A (H3N2) virus coinfection |
| - | + | + | - | + | - | + | - | SARS-CoV-2 & Influenza B virus coinfection |
| - | + | + | - | - | - | + | + | SARS-CoV-2 & RSV coinfection |
| - | - | + | + | + | - | + | - | Influenza A* & B virus coinfection |
| + | - | + | + | + | - | + | - | Influenza A (H1N1) & B virus coinfection |
| - | - | + | + | + | + | + | - | Influenza A (H3N2) & B virus coinfection |
| - | - | + | + | - | - | + | + | Influenza A* & RSV |
| + | - | + | + | - | - | + | + | Influenza A (H1N1) & RSV |
| - | - | + | + | - | + | + | + | Influenza A (H3N2) & RSV |
| - | - | + | - | + | - | + | + | Influenza B & RSV coinfection |

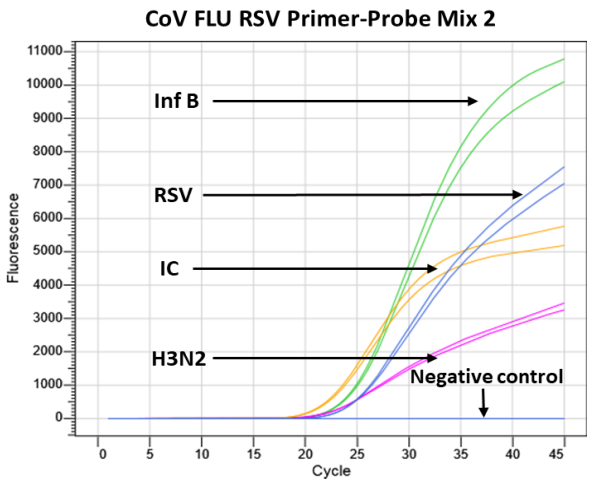
*The infection with non-typable Influenza A is a rare event and it is strongly recommended to send such samples for further identification by additional molecular tools at national laboratories.

**Note**

- Negative results must be combined with clinical observations and patient history. Negative results do not exclude infections because of other variants of these viruses and should not be used as the sole basis for patient management.
- Little is known about epidemiology and outcomes of co-infection between SARS-CoV-2, influenza, or RSV virus. 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 | H1N1 | 26.15 | -- |
| 2 | SARS-CoV-2 | 23.4 | -- |
| 3 | IC | 24.82 | -- |
| 4 | Inf A | 26.05 | -- |

| Sr. No | Target | Ct value | |
|---|---|---|---|
| | | PTC | NTC |
| 1 | Inf B | 24.45 | -- |
| 2 | H3N2 | 22.79 | -- |
| 3 | IC | 22.16 | -- |
| 4 | RSV | 25.57 | -- |

**Note: Image representing probe based Real-Time amplification of different tragets of CoV FLU RSv Primer-Probe Mix 1 and CoV FLU RSv Primer-Probe Mix 2 (Ct values provided in table are for representation only).**

**Performance Evaluation**

**Limit of Detection (LoD) - Analytical Sensitivity**

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® COVID FLU RSV Multiplex Probe PCR Kit was conducted using ATCC Synthetic RNA. 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 LoD of Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit is mentioned below in the chart.

| Target gene | Detected (+) | Limit of Detection |
|---|---|---|
| H1N1 | Ct value ≤ 34 | 1 copy/µL |
| SARS-CoV-2 | Ct value ≤ 36 | 2 copies/µL |
| Inf A | Ct value ≤ 35 | 2 copies/µL |
| H3N2 | Ct value ≤ 30 | 2 copies/µL |
| RSV | Ct value ≤ 34 | 1 copy/µL |
| Inf B | Ct value ≤ 35 | 1 copy/µL |

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.

**Inclusivity - Analytical Sensitivity**

***In silico* analysis**

*In silico* analysis of the Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit oligonucleotides (primers and probes) sequences was performed against all the available sequences in GenBank detected all the reported variants of SARS-CoV-2 virus including the recent circulating JN.1 variant, Beta, Alpha, Omicron, Zeta, Gamma, Delta, Epsilon, Eta, Mu, Eris variants including pango lineages of SARS-CoV-2 virus and also detected the newly identified emerging clades of H1N1, H3N2 and Inf B viruses.

**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.

| | |
|---|---|
| Human coronavirus 229E | *Bordetella pertussis* |
| Human coronavirus HKU1 | *Candida albicans* |
| Human coronavirus NL63 | *Neisseria meningitidis* |
| Human coronavirus OC43 | *Streptococcus Pyogenes* |
| Human rhinovirus 16 | *Haemophilus influenzae* |
| Human metapneumovirus (hMPV) | *Escherichia coli* |
| Enterovirus 68 strain Fermon | *Pseudomonas aeruginosa* |
| Human adenovirus 1 Adenoid 71 | *Staphylococcus aureus* |
| Human parainfluenza virus 1 | *Staphylococcus epidermidis* |
| Human parainfluenza virus 2 | *Chlamydia pneumoniae* |
| Human parainfluenza virus 3 | *Mycoplasma pneumoniae* |
| Middle East respiratory syndrome coronavirus | *Legionella pneumophila* |
| *Candida dubliniensis* | *Candida albicans* |

**Clinical Evaluation**

A total of 460 respiratory clinical samples [85 negative, 75 Influenza A (H1N1)pdm09 positive, 75 Influenza A (H3N2) positive, 75 Influenza B positive, 75 SARS Cov-2 positive and 75 RSVA and B positive] had been tested to evaluate the clinical performance of the Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit. Overall sensitivity and specificity of Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit for all targets obtained was 97.6% and 100% respectively.

| Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit | | Gold standard | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| | Positive | 366 | 0 | 366 |
| | Negative | 9 | 85 | 94 |
| | Total | 375 | 85 | 460 |

| | Estimate | CI 95% |
|---|---|---|
| Sensitivity | 97.6 % | 95.5-98.73 |
| Specificity | 100 % | 95.68-100 |

Individual target specific Sensitivity and Specificity obtained on the clinical evaluation of the Hi-PCR® COVID FLU RSV Multiplex Probe PCR Kit:

| | Influenza A | Influenza A (H1N1)pdm09 | Influenza A (H3N2) | Influenza B | SARS COV-2 | RSV |
|---|---|---|---|---|---|---|
| Sensitivity | 99.33 % | 97.33% | 96.0% | 100 % | 98.67 % | 96.0 % |
| Specificity | 100 % | 100 % | 100% | 100 % | 100 % | 100 % |

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

**Quality Control**

Each lot of Hi-PCR® COVID FLU RSV 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.<br>2. Repeat the analysis of all tests with fresh aliquots of critical reagents. |
| 4. | No signal with positive controls | Incorrect programming of the temperature profile of the thermal cycler | Compare the temperature profile to the manual. |

**Safety Information**

Hi-PCR® COVID FLU RSV 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 samples 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 |
|  | Batch code |  | Temperature limit |
|  | Date of manufacture (YYYY-MM) |  | Consult instructions for use |
|  | Use-by date (YYYY-MM) |  | Catalogue number |

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