# Respiratory Panel Real Time PCR Detection

## SUMMARY AND EXPLANATION OF THE TEST

The respiratory panel real time PCR detection reagent is designed for simultaneous detection of the following pathogens

| | |
|---|---|
| Strep A | Bordetella pertussis |
| RNase P | Chlamydia pneumonia |
| Flu A | Mycoplasma pneumonia |
| Flu B | Adenovirus |
| RSV | Legionella pneumophila |
| Enterovirus | Klebsiella pneumonia group |
| HMPV | Staphylococcus aureus |
| Rhinovirus | Moraxella catarrhalis |
| PIV4 | Bordetella parapert |
| PIV3 | MRSA |
| PIV2 | H Bocavirus |
| PIV1 | Haemophilus influenzae |
| 229E | SC2-N1 |
| HKU1 | SC2-N2 |
| NL63 | Haemophilus influenzae B |
| OC43 | Streptococcus pneumoniae |

## REGENTS AND MATERIALS PROVIDED

| Item | Component | Quantity | | Volume /T |
|---|---|---|---|---|
| | | 100 Tests / Box | 500 Tests / Box | |
| 1 | PCR Mix 1-8 | 1.5 mL/ tube each | 1.5 mL/ tube × 5 each | 14 μL |
| 2 | Enzyme Mix | 850 μL/ tube | 1550 μL/ tube | 1 μL |
| 3 | Positive Control | 100 μL/ tube × 1 | 250 μL/ tube | 5 μL |
| 4 | Negative Control | 100 μL/ tube × 1 | 250 μL/ tube | 5 μL |

## MATERIALS MAY REQUIRED BUT NOT PROVIDED

1. Real-Time PCR Thermocycler compatible with JOE (***VIC or HEX is equivalent to JOE***), ROX, FAM and Cy5 channels.
2. Specimen Collection Containers
3. RNA extraction reagent
4. PCR tubes compatible with Thermocycler.
5. 1.5 mL centrifuge tubes (RNase-Free)
6. Vortex mixer
7. High-speed centrifuge
8. Micropipette (0.5-2 μL, 1-10 μL)
9. Pipette tips with filters
10. Personal protective material

## WARNING AND PRECAUTIONS

1. For research use only. Not for diagnostic procedures.
2. Check the expiration date.
3. Follow Good Laboratory Practices: wear appropriate protective clothing and use disposal gloves and protective eyewear. Do not eat, drink, or smoke in designated work areas. Wash hands thoroughly after handling specimens and kit reagents.
4. The testing workflow must be one-directional to minimize contamination risks (allocate segregated areas for each step): it should be started from RNA Purification Area, then move to the Reaction Setup Area, followed by Amplification and Detection Area. Do not bring samples, equipment, and reagents to the area in which the previous step was performed and always change gloves when changing areas.
5. Regular decontamination of commonly used equipment is recommended, especially for micropipettes and work surfaces.
6. Specimens must be treated as potentially infectious sources as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take appropriate precautions during specimen collection, storage, handling, and disposal in accordance with the guidance from country or region authorities.
7. To ensure optimal performance of the test, always follow appropriate procedures for specimen collection, transport, storage, and processing. Improper procedures may lead to false negative results.
8. Nucleic acids must first be extracted from specimens using a RNA Extraction Kit prior testing. Do not perform a RT-PCR Assay directly using specimens without extraction.
9. Appropriate precautions should be taken to monitor contamination and preserve the purity of kit components and reactions. Avoid microbial and nuclease (RNase/DNase) contamination of specimens and kit components. Avoid the spread of aerosols when handling or uncapping specimens.
10. To minimize cross-contamination, open only one tube at a time in the process.
11. Always change pipette tips between liquid transfers. To minimize cross contamination, it's recommended to use aerosol-barrier pipette tips.
12. Always use disposable gloves and regularly check that they are not contaminated with sample materials. Discard gloves if they become contaminated.

## TRANSPORT AND STORAGE INSTRUCTIONS

Store the kit frozen at -20±5°C and avoid repeated freeze-thaw cycles (less than 5 frozen-thaw times allowed). The product is valid for 6 months, and the expiry date of the product is shown on the label.

## SPECIMEN TYPE and PRESERVATION

**Specimen type:** oropharyngeal swabs, nasopharyngeal swabs and sputum.

**Specimen preservation:** process specimens for viral RNA extraction and nucleic acid detection as soon as possible. Specimens can be stored at 4°C for 24 hours, or frozen at -80°C for 1 year, if not processed immediately. Avoid repeated freeze-thaw cycles during transport and storage of the specimens.

## ASSAY PCR REACTION SETUP (at reagent preparation area)

**A. Reagent Preparation**

To calculate the number (n) of PCR reaction tubes (n= number of specimens to be tested + number of positive controls + number of negative controls), add n x 14 μL of PCR Mix and n x 1 μL of RT-PCR Enzyme Mix to a clean tube, vortex to mix well, centrifuge and aliquot 15 μL of the mix into each PCR reaction tube.

**B. Addition of Viral RNA**

Add 5 μL of viral RNA extracts of the specimen or positive/negative control to each PCR tube according to the reaction preparation table below for testing. Then immediately cap the tube tightly, centrifuge the mixture and move it to the amplification area for RT-PCR detection.

**PCR Reaction Preparation:**

| Component | Volume per test |
|---|---|
| PCR Mix | 14 μL |
| RT-PCR Enzyme Mix | 1 μL |
| Negative Control / Positive Control / RNA of specimen to be tested | 5 μL |
| Total Reaction Volume | 20 μL |

**C. RT-PCR amplification (at Amplification Area, use Applied Biosystems 7500 Real-Time PCR System as an example)**

1. Place the complete PCR reaction tubes into the fluorescent quantitative PCR analyzer and label positive control, negative control and testing specimen on the Thermocycler software.
2. Enter the PCR reaction volume: 20 μL.
3. Select fluorescence detection channels: JOE, ROX, FAM and Cy5 channels.
4. Select PCR cycle parameter setting following the instructions below, save the file and start the PCR run.
5. Analyze the results according to your thermocycler manufacturer's instructions. If the positive and negative control samples do not meet their respective requirements, the PCR run must be repeated.

Note: The positive and negative control can be used directly without purification.

| Cycles | Step | Time | Temperature |
|---|---|---|---|
| 1 | Contamination removal | 2 minutes | 37 ºC |
| 1 | Reverse transcription | 5 minutes | 50 ºC |
| 1 | Initial denaturation | 2 minutes | 95 ºC |
| 40 | Denaturation | 5 seconds | 95 ºC |
| | Annealing/Extension | 20 seconds | 60 ºC |

**QUALITY CONTROL**

Quality control requirements must be performed in conformance with local, accreditation requirements and the user's laboratory's standard quality control procedures.
channel.

**DETERMINATION OF RESULTS**

1. The results can only be determined when the results of both controls meet their requirements.

Multiplex PCR Setup 1

| Channel | Pathogens | Results Interpretation |
|---|---|---|
| Fam | Strep A | |
| JOE | RNase P | |
| ROX | Flu A | |
| Cy5 | Flu B | |

Multiplex PCR Setup 2

| Channel | Pathogens | Results Interpretation |
|---|---|---|
| JOE | RSV | |
| Fam | Enterovirus | |
| ROX | HMPV | |
| Cy5 | Rhinovirus | |

Multiplex PCR Setup 3

| Channel | Pathogens | Results Interpretation |
|---|---|---|
| JOE | PIV4 | |
| FAM | PIV3 | |
| ROX | PIV2 | |
| Cy5 | PIV1 | |

Multiplex PCR Setup 4

| Channel | Pathogens | |
|---|---|---|
| HEX | 229E | |
| FAM | HKU1 | |
| ROX | NL63 | |
| Cy5 | OC43 | |

Multiplex PCR Setup 5

| Channel | Pathogens | Results Interpretation |
|---|---|---|
| Fam | Bordetella pertussis | |
| ROX | Chlamydia pneumonia | |
| Cy5 | Mycoplasma pneumonia | |
| VIC | AV | |

Multiplex PCR Setup 6

| Channel | Bacteria | Results Interpretation |
|---|---|---|
| JOE | Legionella pneumophila | |
| Rox | Klebsiella pneumonia group | |
| FAM | Staphylococcus aureus | |
| Cy5 | Moraxella catarrhalis | |

Multiplex PCR Setup 7

| Channel | Bacteria | Results Interpretation |
|---|---|---|
| JOE | B. parapert | |
| Rox | MRSA | |
| FAM | H Boca | |
| Cy5 | Haemophilus influenzae | |

Multiplex PCR Setup 8

| Channel | Pathogens | Results Interpretation |
|---|---|---|
| JOE | SC2-N1 | |
| FAM | SC2-N2 | |
| ROX | Haemophilus influenzae B | |
| Cy5 | Streptococcus pneumoniae | |

**COMPATIBILITY OF REAL-TIME PCR THERMOCYCLERS**

| Manufacturer | Model |
|---|---|
| Applied Biosystems | 7500 Real-Time PCR System |
| | 7300 plus Real-Time PCR System |

**REFERENCE**

1. National Health Commission & State Administration of Traditional Chinese Medicine. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7). [R]. March 3, 2020

## Symbols used

| Symbol | Meaning |
|---|---|
|  | Manufacturer |
|  | Authorized representative in the European Community |
|  | Catalogue number |
|  | Batch code |
|  | The number of tests |
|  | Caution |
|  | Use by date |
|  | Consult instruction for use |
|  | Date of manufacture |
|  | Storage temperature limit |

Version 250515


Company name: Biomiga, Inc.
Address: 10637 Roselle Street, Suite C, San Diego, CA 92121 USA
Tel: 858-603-3219
Email: info@biomiga.com


Company name: Lotus NL B.V.
Address: Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.
Email: peter@lotusnl.com

**Intended Use:**

For **research use only (RUO)**. Not for use in diagnostic procedures.