RayBio® Coronavirus (SARS-CoV-2) Real Time RT-PCR Nucleic Acid Detection Kit

Catalog #: PCR-COV

User Manual

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ISO 13485 Certified

INTRODUCTION

The Coronavirus (SARS-CoV-2) Real Time RT-PCR Nucleic Acid Detection Kit is based on the PCR method which uses a fluorescent probe and a specific primer to detect three specific regions within the novel coronavirus (SARS-CoV-2) nucleocapsid protein N gene. This molecular panel aids in the detection of viral RNA from SARS-CoV-2, the causative agent of COVID-19. The kit includes 3 primer-probe sets corresponding to those used in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (cat no. 2019-nCoVEUA-01).

Primer-probe sets N1 and N2 detect SARS-CoV-2 specifically, with no expected false positives from other coronaviruses or human microflora, while N3 detects SARS-CoV-2 as well as human SARS coronavirus, but no expected false positives from other coronaviruses or human microflora. The kit also includes a primer-probe set specific to the to the human housekeeping gene, ribonuclease P (RNP), are included to serve as an internal reference to monitor sample collection, RNA extraction, and amplification.

PACKAGING SPECIFICATIONS

20 tests/box

PURPOSE

This kit is used for the qualitative *in vitro* detection of novel coronavirus (SARS-CoV-2) nucleic acid in patient sera and respiratory specimens (including pharyngeal swabs and alveolar lavage fluid samples).

KIT COMPONENTS

Component	Ingredients	Specification	Quantity
Primer and Probe N1 Solution	Primers & probe for N1	140 µL / tube	1 tube
Primer and Probe N2 Solution	Primers & probe for N2	140 µL / tube	1 tube
Primer and Probe N3 Solution	Primers & probe for N3	140 µL / tube	1 tube
Primer and Probe RP Solution	Primers & probe for Human RNAse P	140 µL / tube	1 tube
PCR Reaction Solution	Buffer, dNTP's	1200 µL / tube	1 tube
PCR Enzyme Mix	Reverse transcriptase (RT) enzyme, DNA polymerase	160 µl / tube	1 tube
Positive Control	Plasmid DNA containing target gene (Nucleocapsid protein) + plasmid DNA containing internal control gene fragment (RNP)	480 µL / tube	4 tubes
Negative Control	Nuclease-free water	480 µL / tube	1 tube

Note: Do not mix reagents from different lots.

STORAGE AND EXPIRATION

The kit can be stored at -20°C for a period of 12 months prior to opening. After opening, the reagents are valid for 6 months if stored at -20°C. Up to 3 freeze-thaw cycles are permitted while retaining activity.

REQUIRED MATERIALS (NOT INCLUDED)

Fluorescence qPCR instrument capable of reading FAM or equivalent channels (494 nm maximum absorption, 518 nm maximum emission).
Vortex Mixer
Microcentrifuge
Pipettes
Sterile nuclease-free pipette tips and microfuge tubes
RNA extraction kit (e.g., Qiagen RNeasy® Mini Kit, EZI DSP Virus Kit, or similar)

SAMPLE REQUIREMENTS

1. Sample types: total RNA extracts from throat swab or alveolar lavage fluid. Note: collection of samples should be conducted according to the relevant guidelines of your governing body.
2. Samples should be regarded as a potential source of infection. Sample handling should be performed in a microbiological and biomedical laboratory with a biosafety label to protect the operator from possible exposure during work.

GENERAL CONSIDERATIONS

1. To prevent contamination of PCR reactions, clean and decontaminate all working surfaces, centrifuges, pipets, and other equipment with 10% bleach or RNase Away®.
2. Conduct sample processing and RNA extraction in a separate area from the PCR assay setup. Additionally, care should be taken to avoid contamination of samples and reactions with RNA from used PCR plates. It is thus recommended to place used PCR plates in a sealed bag and discard them appropriately. Never open a used PCR plate or place it in the same area as the PCR instrument.
3. To minimize cross-contamination between experimental samples, disposable pipettes and pipette tips are recommended.

TESTING METHOD

1. Sample Processing (Sample Processing Area)

1.1. Use an RNA sample preservation solution for virus inactivation and RNA preservation.

1.2. A nucleic acid extraction or purification kit, Trizol-based extraction may be used to extract the nucleic acid. Other commercially available virus nucleic acid extraction kits can also be used.

2. Assay Assembly (PCR Assay Setup Area)

2.1. **Thaw reagents:** Remove the PCR Reaction Solution and the PCR Enzyme Mix from the kit, and fully thaw to room temperature. Mix gently by pipetting. Briefly centrifuge to collect contents at bottom of vial.

2.2. **Calculate number of reactions needed:** The number of reactions to be prepared per PCR run maybe calculated by (# of samples to be tested +2) x 4. Adding 2 to the number of samples to be tested takes positive and negative controls tests into account. It is recommended to include 1 positive and 1 negative control with each experiment. Refer to Table 1 for a summary of reaction components included in each well. NOTE: it will be necessary to make excess reactions to account for pipetting error.

2.3. **Prepare PCR Master Mix:** Each reaction should contain 12.5 µl PCR Reaction Solution and 1 µl Enzyme Mix. To calculate the total volume necessary for the run, multiply the volumes of each component by the number of reactions calculated in 2.2. Mix the PCR Reaction Solution and Enzyme mix together to prepare a Master Mix.

2.3.1. Example: for 10 samples, 48 reactions are needed which requires 600 µl PCR Reaction Solution and 48 µl Enzyme Mix, for a total of 648 µl PCR Master Mix. Add the appropriate volume of each component into a tube and mix gently by pipetting.

2.4. **Divide PCR Master Mix:** Dispense PCR Master Mix evenly into 4 tubes. Each tube is designated for one of the four Primer Solutions. From the example in 2.3, each tube would receive 162 µl PCR Master Mix.

2.5. **Add Primer and Probe N1 Solution:** The total required volume of Primer 1 Solution is (# of reactions x 1.5 µl). Add the calculated volume of Primer 1 Solution to one of the PCR Master Mix tubes from step 2.4. Mix gently by pipetting.

2.6. Repeat step 2.5 for Primer and Probe Solutions N2, N3, and RP.

Table 1: Reaction Components for Samples and Controls.

Component	Positive Control Reaction	Negative Control Reaction	Sample Reaction
PCR Reaction Solution	12.5 µl	12.5 µl	12.5 µl
PCR Enzyme Mix	1 µl	1 µl	1 µl
Primer Solution	1.5 µl	1.5 µl	1.5 µl
Positive Control	5 µl	--	--
Negative Control	--	5 µl	--
Sample	--	--	5 µl

Total Volume	**20µl**	**20µl**	**20µl**

3. Sample Loading (PCR Assay Setup Area)

3.1. Set up the PCR Plate: Pipette 15 µl of each reaction mixture from step 2.6 into the PCR plate according to the layout in Figure 1.

3.2. Add 5 µl of sample to each well and pipette up and down at least 5 times to mix.

3.3. Seal the plate or tubes tightly.

3.4. Centrifuge the plate or tubes for 30 seconds at low speed. Note: The sealed PCR reaction tubes can be stored at 2-8°C for up to 4 hours before the “PCR Amplification” step.

	1	2	3	4	5	6	7	8	9	10	11	12
A	N1	N2	N3	RP	N1	N2	N3	RP	N1	N2	N3	RP
B	N1	N2	N3	RP	N1	N2	N3	RP	N1	N2	N3	RP
C	N1	N2	N3	RP	N1	N2	N3	RP	N1	N2	N3	RP
D												
E												
F												
G												
H												

Figure 1. Example of a RT-PCR plate layout with 6 samples, 1 positive control (white wells) and 1 negative control (gray wells).

4. PCR Amplification (PCR Assay Setup Area)

4.1 **Sample setup:** Set the sample number, targets, negative control and positive control accordingly to your plate setup.

4.2 **Fluorescence Channel Selection:** Select FAM or equivalent channel. The reference fluorescence (passive reference) will not be used and should be set to “none.”

4.3 Set reaction conditions according to Table 2. The reaction volume is set to 20 µl.

Table 2: Real Time RT-PCR Program

Step		Temperature (°C)	Time	Temperature Ramp Rate	Number of Cycles
Stage 1	Reverse transcription	55	10 min	1.6°C/sec	1
Stage 2	Pre-denaturation	95	5 min	1.6°C/sec	1
Stage 3	Denature	95	10 s	1.6°C/sec	45
	Anneal, extend, detect fluorescence	55	30 s	1.6°C/sec	

4.4 Save the file and run program.

5. Result and Analysis

The positive and negative control reactions PCR reactions are considered valid if the negative and positive controls meet the criteria listed in Table 3. The PCR reaction is invalid if 1) the positive control does not have logarithmic growth or the Ct < 35 or 2) the negative control has a Ct $\geq$ 40. If the reaction is invalid, the measurement of all samples in this experiment should be repeated.

Table 3. Validation of PCR reactions with quality controls

Target	Positive Control	No Template Control
N1	Ct ≤ 35	Ct ≥ 40 or no Amplification
N2	Ct ≤ 35	Ct ≥ 40 or no Amplification
N3	Ct ≤ 35	Ct ≥ 40 or no amplification

RP	Ct ≤ 35	Ct ≥ 40 or no amplification

6. INTERPRETATION OF TEST RESULTS

The PCR reaction results are explained according to Tables 4 and 5.

Table 4. Interpretation of Individual PCR Reactions

PCR reaction results	Ct
+	< 40
-	No amplification, or Ct > 45
Suspect	40 ≤ Ct < 45

Suspect Ct values may indicate poor PCR performance due to contaminants or low RNA amount. Retesting is recommended to confirm results.

Table 5. Interpretation of Sample Test

Result	N1	N2	N3	RP
Inconclusive	If only 1 or 2 targets are positive			+/-
Negative for COVID-19	-	-	-	+
Positive for COVID-19	+	+	+	+/-
Invalid	-	-	-	-

Positive Result: the sample contains the target genes.

Negative Result: the sample does not contain the target genes.

Suspect/Invalid Result: the sample nucleic acid should be re-extracted and re-run. Very low RNA was present. If N3 and RP are positive, it may indicate presence of other coronavirus genes.

LIMITATIONS OF DETECTION METHOD

1. The results of this assay are applicable for research purposes only and are not intended for clinical diagnosis of patients.
2. A “negative result” may be a false negative (i.e., a sample contains genetic material of SARS-CoV-2). Possible causes of a false negative result:
   - 2.1. Faulty sample collection, processing, transportation, or low sample concentration
   - 2.1 Variations in the target sequence of the 2019-nCoV novel coronavirus or sequence changes caused by other reasons
   - 2.2 Improper reagent storage
   - 2.3 Other unverified interferences or PCR inhibitors
   - 2.4 Cross-contamination during sample processing

PRODUCT PERFORMANCE INDEX

1. **Limit of Detection:** the minimum detection limit of this kit is 40 copies / mL.
2. **Repeatability:** Precision testing showed that the coefficient of variation of the precision Ct values within this kit lot are ≤ 5%.
3. **Accuracy**: The kit was tested with a third-party sourced positive reference product and the compliance rate was 100%. The kit was tested with a third-party sourced negative reference product and the compliance rate was 100%.

REFERENCES

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