RayBio® COVID-19
1-Step High Throughput PCR Kit

Catalog #: PCR-COV-HTOS

User Manual

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RayBiotech
Empowering your proteomics

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INTRODUCTION

The RayBio® COVID-19 1-Step High Throughput PCR Kit is based on the PCR method which uses a fluorescent probe and a specific primer to detect two 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 also contains a proprietary buffer which enables the user to use crude saliva or throat swabs directly in their PCR reactions. By simply mixing the 1-Step Nucleic Acid Stabilization Buffer with the sample, the viral RNA is immediately stabilized, and interfering components of the saliva matrix are inactivated. This eliminates the need for laborious, costly RNA extraction and purification steps, drastically improving upon the traditional workflow of PCR, while removing potential sample loss and contamination. This kit utilizes an alternative method of data analysis than most traditional PCR assays. Instead of utilizing Ct values, which can vary significantly due to sample matrix effects, this assay uses the ratio of the fluorescence of the sample at the beginning of the run versus the fluorescence at the end at an elevated temperature. This method of analysis can help differentiate from signal resulting from a low-abundance target in a difficult sample medium from a non-specific signal in a complex sample media (such as crude saliva).

The kit includes 2 primer-probe sets (included in the PCR Reaction Solution) 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.

PACKAGING SPECIFICATIONS

100 tests/box

PURPOSE

This kit is used for the qualitative *in vitro* detection of novel coronavirus (SARS-CoV-2) nucleic acid in saliva or throat swab samples.

KIT COMPONENTS

Component	Ingredients	Specification	Quantity
PCR Reaction Solution	Buffer, dNTP's, Primers and Probes	1400 µL / tube	1 tube
PCR Enzyme Mix	Reverse transcriptase (RT) enzyme, DNA polymerase	100 µl / tube	1 tube
Positive Control	Plasmid DNA containing target gene (Nucleocapsid protein), 8 copies per µL	480 µL / tube	1 tube
Negative Control	Nuclease-free water	480 µL / tube	1 tube
1-Step Nucleic Acid Stabilization Buffer	Buffer	10mL / bottle	1 bottle

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. The 1-Step Nucleic Acid Stabilization Solution can be stored at room temperature.

REQUIRED MATERIALS (NOT INCLUDED)

- Fluorescence qPCR instrument capable of reading SYBR or equivalent channels, (494 nm maximum absorption, 518 nm maximum emission), and ROX or equivalent channels (578 nm maximum absorption, 604 nm maximum emission).
- Vortex Mixer
- Microcentrifuge
- Pipettes
- Sterile nuclease-free pipette tips and microfuge tubes

SAMPLE REQUIREMENTS

1. Sample types: throat swabs stored in VTM (viral transport medium), nasopharyngeal swabs stored in VTM, or saliva.
2. Samples should be regarded as a potential source of infection. Sample handling should be performed in a microbiological and biomedical laboratory with an appropriate 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 in a separate area from the PCR assay setup. Additionally, care should be taken to avoid contamination of samples and reactions with DNA 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.
4. To avoid RNA degradation, it is recommended to add the 1-Step Nucleic acid Stabilization Buffer to the sample immediately after collection. Alternatively, the sample may be stored on ice for 1 hour prior to adding Stabilization Buffer.

TESTING METHOD

1. Sample Processing (Sample Processing Area)

1.1. **FOR SALIVA**

1.1.1. Mix 100µL of saliva in a new tube with 100µL of the 1-Step Nucleic Acid Stabilization Buffer until the solution is homogenous. Centrifuge the sample using a benchtop centrifuge for 30 seconds to pellet the debris. Transfer the supernatant to a new tube and store at 4ºC for up to 24 hours before use or freeze for long term storage.

1.2. **FOR SWABS**

1.2.1. For swabs, vortex or mix the swab/VTM tube briefly to ensure the VTM is well mixed. In a sterile microfuge tube, mix 100 µL of the 1-Step Nucleic Acid Stabilization Buffer with 100 µL of the VTM the swab is stored in. Cap and vortex for 30 seconds. This solution will be used in Step 3.2. NOTE: Before storing the remaining solution in the tube with the swab, briefly centrifuge the tube to collect the solution at the bottom of the tube. A precipitate may be present at the bottom of the tube. Store at 4ºC for up to 24 hours before use or freeze for long term storage.

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). Adding 2 to the number of samples 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 is advised to make excess reactions to account for pipetting error.

2.3. **Prepare PCR Master Mix:** Each reaction should contain 14 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. Combine the PCR Reaction Solution and Enzyme Mix together to prepare a Master Mix.

Table 1: Reaction Components for Samples and Controls.

Component	Positive Control Reaction	Negative Control Reaction	Sample Reaction
PCR Reaction Solution	14 µl	14 µl	14 µl
PCR Enzyme Mix	1 µl	1 µl	1 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 (prepared in Step 2.3) into the PCR plate.

3.2 Add 5 µl of sample (prepared in Step 1) 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 (500xg). Note: The sealed PCR reaction tubes can be stored at 2-8°C for up to 4 hours before the "PCR Amplification" step.

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 SYBR or equivalent channel for each sample and label the channel as "SARS-COV-2". Also add the ROX channel and label the channel as "normalization" for each sample. 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		Detect Fluorescence	Temperature (°C)	Time	Temperature Ramp Rate	Number of Cycles
Stage 1	Reverse transcription	No	50	2 min	1.6°C/sec	1
Stage 2	Pre-denaturation	No	95	10 min	1.6°C/sec	1
Stage 3	Denature	No	95	5 s	1.6°C/sec	40
	Anneal, extend, detect fluorescence	Yes	60	30 s	1.6°C/sec	
Stage 4	Detect final fluorescence	Yes	90	30 s	1.6°C/sec	1

4.4 Setup a melt curve step after stage 3, from 70ºC-95ºC, with at least 0.15ºC intervals to determine the Tm of the amplification products.

4.5 Save the file and run program.

5. Results and Analysis

5.1 To extract the Fluorescence data, export the data from the QuantStudio software making sure that the "multicomponent data" box is checked and export the file. Open the Excel file and select the "multicomponent data" tab. Scroll down until you find the data collected in cycle 10 and copy the data for each well to be analyzed to a new file. Continue to scroll down the sheet until you arrive at the data for cycle 41 (the final reading which was collected at 90ºC). Copy the data for each well to be analyzed to the file with the cycle 10 data. NOTE: if using an instrument other than the QuantStudio 5, please consult the manufacturer's specifications for extraction of total fluorescence during a specified cycle.

5.2 The analysis of the assay is accomplished by obtaining the fluorescence value at cycle 10 of the PCR and the fluorescence detected at the final reading (stage 4). Divide the final fluorescence value (F) by the cycle 10 fluorescence value (10) to obtain a ratio value (F/10 ratio). The same should be done for the ROX channel to obtain the normalization ratio. The F/10 ratio is then divided by the normalization ratio to obtain the final ratio.

5.3 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 reactions are invalid if 1) the positive control has a final ratio of less than 1.0, or 2) the negative control has a final ratio greater than 1.0. 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
COVID-19	Final ratio > 1.0	Final ratio < 1.0

INTERPRETATION OF TEST RESULTS

The PCR reaction results are explained according to Table 4.

Table 4. **Interpretation of Individual PCR Reactions**

PCR Reaction Results	Final Ratio
+	> 1.0
Inconclusive	0.88 ≥ 1.0
-	No amplification, or final Ratio < 0.88

Positive Result: the sample contains the target genes for SARS-COV-2.

Inconclusive Result: the sample should be rerun in triplicate, and the 3 final ratios averaged. If the average final ratio is ≥ 0.88, the sample is positive. If the average final ratio is < 0.88, the sample is negative.

Negative Result: the sample does not contain the target genes or is below the detectable limit for SARS-COV-2.

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
   2.2 Variations in the target sequence of the 2019-nCoV novel coronavirus or sequence changes caused by other reasons
   2.3 Improper reagent storage
   2.4 Other unverified interferences or PCR inhibitors
   2.5 Cross-contamination during sample processing

PRODUCT PERFORMANCE INDEX

1. **Limit of Detection:** the minimum detection limit of this kit is 20 copies/reaction or 4 copies/μL of sample input.
2. **Repeatability:** Precision testing showed that the coefficient of variation of the precision 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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3. Elien, of Mo Ë S, Leen Vijgen, E LS Keyaerts, et Al. A Novel pancoronavirus RT-the PCR Assay: Frequent Detection of Human coronavirus NL63 in Children Hospitalized with Respiratory tract infections in Belgium. The BMC Infectious Diseases, 2005, 5.

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