RayBio® COVID-19 Rapid Isothermal PCR Kit

Detect SARS-CoV-2 RNA in 30 minutes
without a PCR instrument

User Manual Version 1.2
November 3rd, 2020

Catalog numbers: **RT-LAMP-B-25 (25 tests)**
RT-LAMP-B-100 (100 tests)

RayBiotech | *Empowering your proteomics*

Providing you with high quality products and services since 2001

Tel: (770) 729-2992
www.raybiotech.com

Fax: (770) 206-2393
info@raybiotech.com

RayBiotech Life, Inc.

RayBio® COVID-19 Rapid Isothermal PCR Kit

TABLE OF CONTENTS

I. Introduction

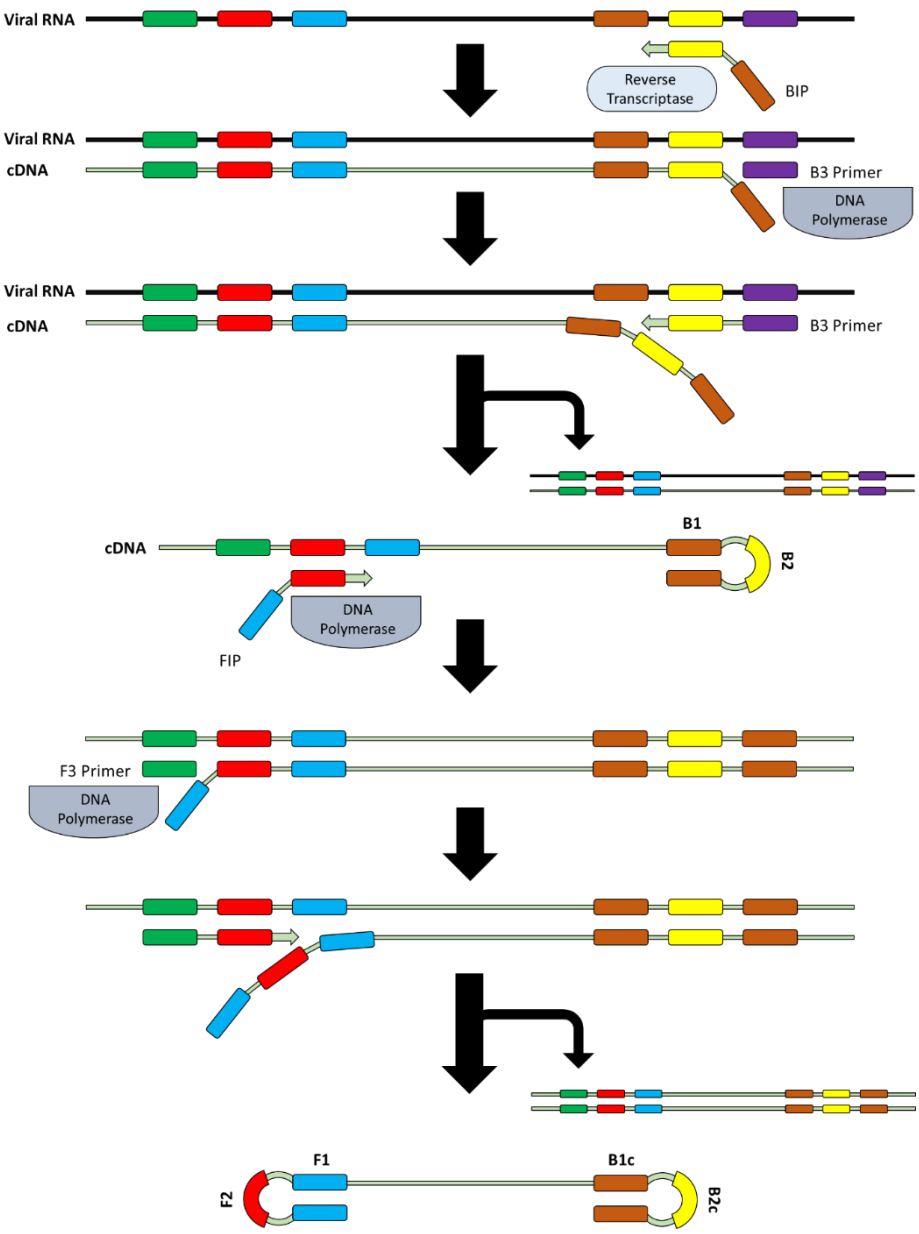
The SARS-CoV-2 virus is responsible for coronavirus disease 2019 (COVID-19), which is associated with a wide range of symptoms (e.g., coughing, muscle pain, headache, sore throat). For some infected individuals, COVID-19 causes mild or no symptoms even though they are still contagious. For others, COVID-19 can lead to prolonged illness, hospitalization, and even death. The identification of infected individuals is paramount in stopping the spread of COVID-19. Viral RNA detection via RT-PCR requires specialized training, hours to complete, and advanced equipment. With RayBiotech's COVID-19 Rapid Isothermal PCR Kit, the viral RNA can be detected in 30 minutes with minimal training and only a heat block. This RayBio® kit utilizes reverse transcription loop-mediated isothermal amplification (RT-LAMP), which amplifies SARS-CoV-2 RNA faster than traditional RT-PCR with a colorimetric endpoint readout. Thus, detection of viral RNA can be performed by eye or with a plate reader capable of measuring absorbance at 570 nm and 650 nm. This kit's master mix includes UDG and dUTP to reduce the risk of carryover contamination. The assay is compatible with extracted RNA and is pH-independent.

II. Kit Overview

RayBio® COVID-19 Rapid Isothermal PCR Kit	Amplification of the SARS-CoV-2 RNA gene encoding for the nucleocapsid protein
Amplification Method	One-step RT-LAMP (reverse transcription loop-mediated isothermal amplification)
Colorimetric Assay	Negative for SARS-CoV-2: Purple (570 nm) Positive for SARS-CoV-2: Blue (650 nm)
Reaction Volume	25 µl
Sensitivity	25 copies/µl
Assay Duration	30 minutes

III. How it works

The RayBio® COVID-19 Rapid Isothermal PCR Kit utilizes reverse transcription loop-mediated isothermal amplification (RT-LAMP), which amplifies specific RNA regions at a single specific temperature. First, RNA of the SARS-CoV-2 nucleocapsid gene is reverse transcribed into cDNA using specific outer primers (F3 and B3, see figure below). Once cDNA is generated, the specific inner primers (FIP and BIP) anneal, extend and fold back on themselves, forming a dumbbell structure to enable continuous cDNA amplification of the selected regions without the need for thermal cycling. This kit uses six primer-probe sets specific to the SARS-CoV-2 gene encoding for the nucleocapsid protein.



IV. Materials Provided

Item	Description	25 Tests	100 Tests
1	RayBio® COVID-19 Rapid Master Mix	375 µl	1.50 ml
2	SARS-CoV-2 Primer Mix	125 µl	500 µl
3	Synthetic SARS-CoV-2 Nucleocapsid RNA (1000 copies/µl)	10 µl	10 µl
4	Ultra-pure DNase- and RNase-free Water	1 ml	1 ml
5	Manual	1	1

Kit ships on dry ice. Upon receipt, all components of the RayBio® COVID-19 Rapid Isothermal PCR Kit should be stored at -20°C. If stored properly, the kit is good for up to 6 months.

The number of freeze-thaw cycles for the Master Mix (Item 1) and SARS-CoV-2 Synthetic RNA (Item 3) should be minimized. Upon thawing for the first time, these reagents should be aliquoted appropriately for future use and stored back at -20°C.

Additional Materials Required

- RNA purification kit to prepare RNA from collected samples. 5 µL of purified RNA in ultra-pure water is required per well of the RayBio® COVID-19 Rapid Isothermal PCR Kit.
- Purified RNA. The recommended minimum amount of total purified RNA per well is 100 ng.
- Pipettes with filtered, disposable tips capable of accurately dispensing 5 µL – 100 µL.
- 0.2 ml DNase-free and RNase-free PCR tubes, strips, or plates with caps
- Centrifuge capable of spinning 0.2 ml PCR tubes, strips, or PCR plates at 1000 g
- Dry heat bath with heated lid capable of heating to 80°C with appropriate metal block to hold 0.2 ml PCR tubes ***or*** a PCR thermal cycler.
- Plate reader capable of UV/Vis OD measurement at 570 nm and 650 nm (optional)

V. Assay Protocol

Preparation of Reagents

- Thaw all reagents on ice prior to the assay.
- Spray dedicated clean area with solutions to eliminate RNases and DNA (*see "Important Note!" below*)
- Use filtered pipette tips to prevent cross-contamination.
- If the kit will not be used in its entirety at one time, aliquot the reagents after the first thaw and store at -20°C. Minimize the number of freeze-thaw cycles to maintain the integrity of the reagents.

Important note! *Because this kit is very sensitive, it is highly recommended that the reagents are prepared in a dedicated clean area that is separate from the sample collection and testing sites. The kit contains UDG and dUTP that can reduce carry-over contamination amplified with dUTP.*

Assay Procedure

1. Before every sample preparation, spray the dedicated clean area with solutions to eliminate RNases and DNA.

2. Obtain enough 0.2 ml PCR tubes for the number of samples to be run, including an additional tube for a positive control, and 1 tube for a negative control. *All reactions should be kept on ice during preparation steps.*

   Note: Analyzing controls and samples in duplicate or triplicate is recommended.

3. Prepare positive control working solution:
   - Pipette 5 µL of the provided SARS-CoV-2 Synthetic Nucleocapsid RNA into a new 0.2 ml PCR tube.
   - Dilute with 35 µL of Ultra-pure water.
   - Mix well by pipetting up and down 3 – 5 times to generate 125 copies/µL of synthetic RNA ("Working Stock").
   - Place on ice until ready to use.

   Note: For future use, the "Working Stock" of synthetic RNA can be aliquoted and stored at -20°C. Minimize the number of freeze-thaw cycles to maintain the integrity of the reagent.

4. Prepare a Working Reaction Mixture by adding the following components together (per sample or control):

    - 15 µL of RayBio® COVID-19 Rapid Master Mix
    - 5 µL of SARS-CoV-2 Primer Mix

    Note: Analyzing samples in duplicate or triplicate is recommended.
    Note: A Working Reaction Mixture for all controls and samples can be prepared at one time.

5. Place 20 µL of the Working Reaction Mixture into a separate tube, then add 5 µL of the following for a final volume of 25 µL:
    - **Sample:** Add 5 µL of purified RNA in Ultra-pure water.
    - **Negative control:** Add 5 µL of Ultra-pure water.
    - **Positive control:** Add 5 µL of the "Working Stock" of synthetic RNA. The final viral RNA concentration of this positive control is 25 copies/µL.

6. Mix the reaction by pipetting up and down 5 – 10 times. Cap the tubes once mixing is complete. If using a PCR plate, seal well with PCR film to prevent cross-contamination.

    *Note: Vortexing the PCR plate or strip is **not** recommended as this can cause cross contamination between samples. Vortexing or violent mixing of samples may also affect the integrity of the samples and kit reagents.*

7. Spin the tubes or plate at 1000 g for 1 min to remove bubbles from the bottom of the tubes.

8. Let sit at room temperature for 5 minutes.

    Note: This step eliminates potential contaminating DNA, thus eliminating false positives that occur from carryover contamination.

9. Put the samples in a heat block with heated lid set to 60°C or a PCR thermal cycler set at 60°C for 30 minutes.

    Note: Because RT-LAMP amplifies the DNA quickly, all samples with at least 25 copies/µL of the SARS-CoV-2 virus will eventually reach the saturated platform stage. Therefore, 30 minutes is the optimal incubation length to accurately assess the presence of viral RNA in the samples against the positive and negative controls.

10. Remove samples from heat block or PCR thermal cycler. Results are stable at room temperature for up to 12 hours. (It is not necessary to inactivate the reaction.)

11. The presence of viral RNA in the sample can then be determined by eye or with a plate reader capable of measuring absorbance at 570 nm and 650 nm (see Data Interpretation).

VI. Data Interpretation

The RT-LAMP colorimetric master mix contains a purple dye that changes to blue as the SARS-CoV-2 cDNA is amplified.

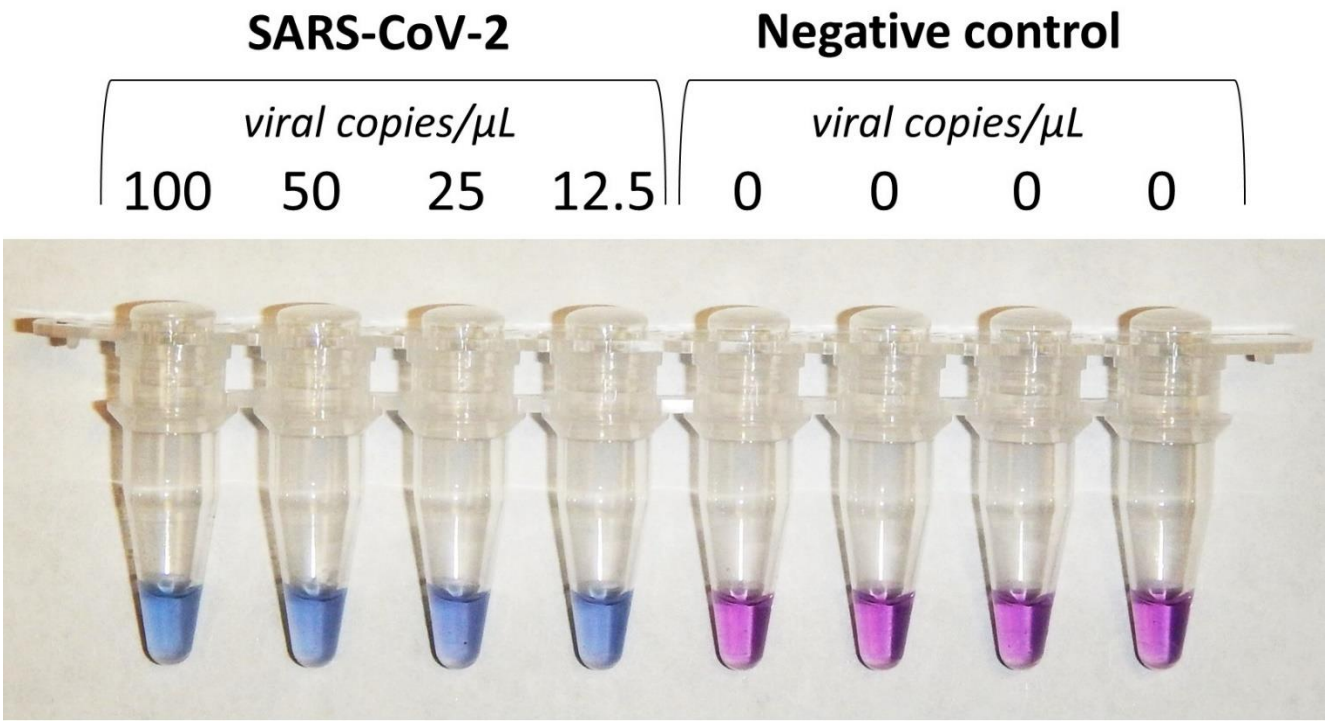


Figure (above). The sample color changes from purple to blue when the SARS-CoV-2 cDNA is amplified

The results can be interpreted using the following methods:

1. **By eye:** Compare the sample color to the negative controls (purple). A sample that is as blue as the positive control indicates that SARS-CoV-2 viral RNA is present (positive result), while purple – that is, the lack of a blue hue – indicates that the sample does ***not*** have SARS-CoV-2 viral RNA (negative result).

2. **With a plate reader:** Depending on your instrument, the reaction may need to be transferred to a new plate or tube. Since plate wells require more volume than the PCR tube, we recommend merging the sample from duplicate or triplicate tubes into the same well on the plate to get a more accurate reading if required. Measure the purple and blue absorbances at 570 nm and 650 nm, respectively. Divide the sample optical density (OD) at 570 nm by the OD at 650 nm (OD650 nm / OD570 nm). A ratio over 1.0 indicates that SARS-CoV-2 viral RNA is present (positive result), while a ratio less than 1.0 indicates that the sample does ***not*** have SARS-CoV-2 viral RNA (negative result). See next page for representative data.

Note: A sample having a blue hue with an OD 650/570 nm ratio close to 1.0 should be considered as "suspect." It is recommended that the sample is run again with a higher total RNA content for confirmation.

Below is a table of representative data using synthetic viral RNA. As noted above, all positive samples will eventually reach the saturated platform stage. Here, samples with ≥ 12.5 viral copies per µL in the reaction reached saturation.

Correlation of RNA copies to OD 650/570 nm Ratio**

RNA copies / µL	OD 650/570 nm*
0	0.6
3.125	0.603
6.25	0.874
12.5	1.21
25	1.189
50	1.202
100	1.208
200	1.219

* Averaged across triplicate wells.
** Synthetic viral RNA spiked into a human RNA sample from an oropharyngeal swab

VII. Troubleshooting guide

Problem	Cause	Recommendation
No Color Change in Positive Control	No RNA was added	Ensure positive control was prepared correctly and was added to the reaction
	Positive control underwent too many freeze-thaw cycles	If entire kit will not be run at one time, aliquot reagents appropriately for future use to minimize the number of freeze-thaw cycles
	Reaction was not prepared correctly	Double check the reaction volume and ensure that the samples were mixed well
	The reaction was not performed at the appropriate temperature	Check calibration of the instrument used to maintain temperature
	The reaction was not long enough	Allow the reaction to run for 30 minutes
	Improper storage of kit	Store kit at -20˚C
Color Change in Negative Control	Sample Contamination	Only use tubes or lids that have not been used before. Change pipette tips between sample wells. Be careful to maintain samples in their own wells during mixing. Use fresh water for the negative control. Perform step 8.
	The reaction was not performed at the appropriate temperature	Check calibration of the instrument used to maintain temperature
	The reaction occurred for too long	Rerun the assay; allow the reaction to run for the recommended 30 minutes
	Improper storage of kit	Store kit at -20˚C
	Kit reagents underwent too many freeze-thaw cycles	If entire kit will not be run at one time, aliquot reagents appropriately for future use to minimize the number of freeze-thaw cycles

VIII. Note

RayBio® is the trademark of RayBiotech Life, Inc.

This product is intended for research only and is not to be used for clinical diagnosis. Our products may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech Life, Inc. Under no circumstances shall RayBiotech be liable for any damages arising out of the use of the materials.

Products are guaranteed for six months from the date of purchase when handled and stored properly. In the event of any defect in quality or merchantability, RayBiotech's liability to buyer for any claim relating to products shall be limited to replacement or refund of the purchase price.