RayBio® SARS-CoV-2 RNA Isothermal Amplification Colorimetric Assay Kit

Detect SARS-CoV-2 RNA in 20 minutes
without a PCR instrument

User Manual Version 3.0
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Catalog numbers: **RT-LAMP-25 (25 tests)**
RT-LAMP-100 (100 tests)
RT-LAMP-500 (500 tests)

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RayBiotech Life, Inc.

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II. Introduction

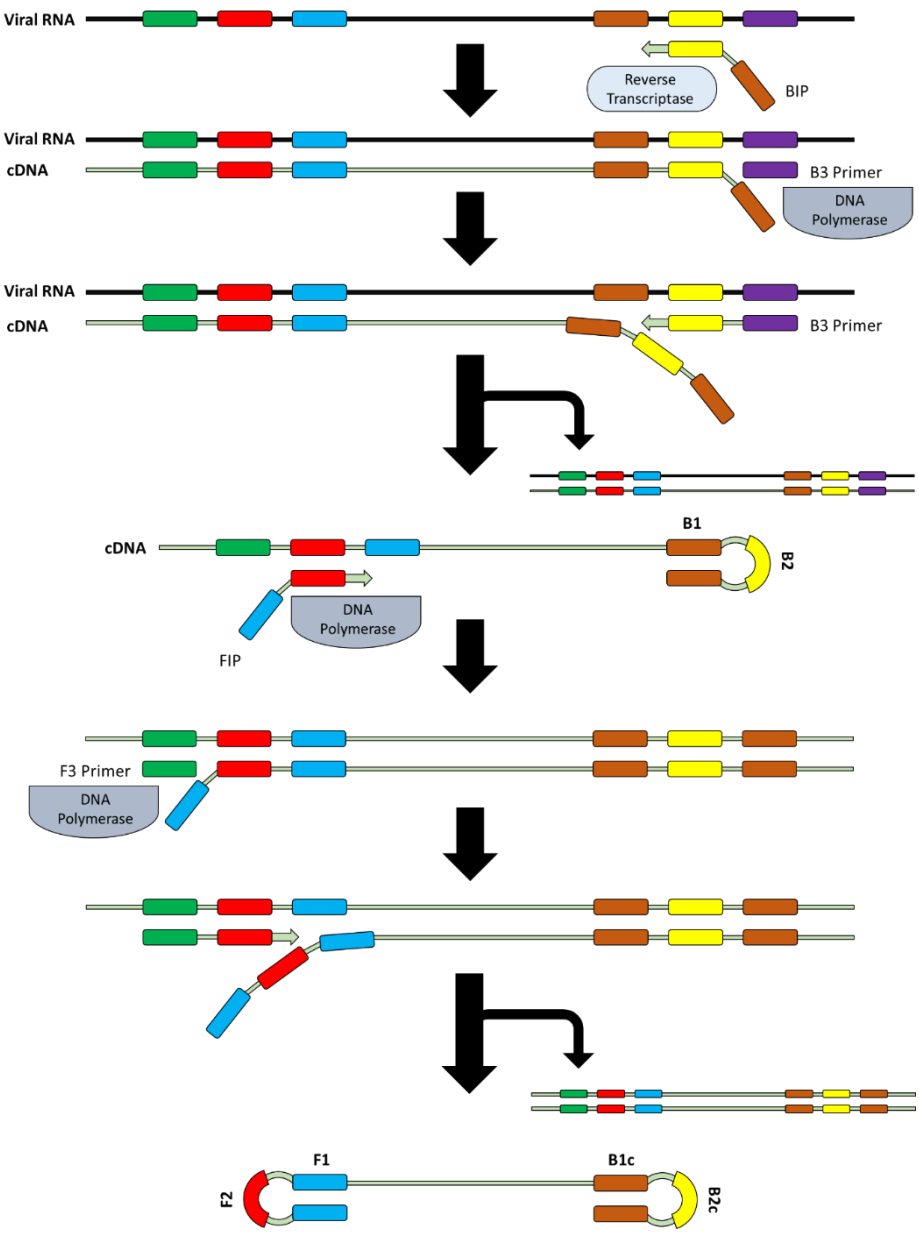
The SARS-CoV-2 virus is responsible for coronavirus disease 2019 (COVID-19), which is associated with a wide range of symptoms (e.g., coughing, muscle pain, headache, sore throat). For some infected individuals, COVID-19 causes mild or no symptoms even though they are still contagious. For others, COVID-19 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 SARS-CoV-2 RNA Isothermal Amplification Colorimetric Assay Kit, the viral RNA can be detected in 20 minutes with minimal training and only a heating block. This RayBio® assay 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 440 nm and 560 nm.

III. Kit Overview

RayBio® SARS-CoV-2 RNA Isothermal Amplification Colorimetric Assay Kit	Amplification of 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: Pink Positive for SARS-CoV-2: Yellow
Reaction Volume	25 µl
Sensitivity	25 copies/µl
Assay Duration	20 minutes

IV. How it works

The RayBio® SARS-CoV-2 RNA Isothermal Amplification Colorimetric Assay Kit utilizes reverse transcription loop-mediated isothermal amplification (RT-LAMP), which amplifies specific RNA regions using only a heating block. 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	500 Tests
1	RayBio® SARS-CoV-2 RNA RT-LAMP Colorimetric Master Mix	375 µl	1.50 ml	1.50 ml x 5
2	SARS-CoV-2 Synthetic Nucleocapsid RNA (1000 copies/µl)	10 µl	10 µl	10 µl x 5
3	Ultra-pure DNase- and RNase-free Water	2 ml	2 ml	2 ml x 5
4	Manual	1	1	1

Upon receipt, all components of the RayBio® SARS-CoV-2 RNA Isothermal Amplification Colorimetric Assay 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 SARS-CoV-2 Synthetic RNA should be minimized. Upon thawing for the first time, the SARS-CoV-2 Synthetic RNA should be aliquoted appropriately for future use and stored at -20˚C.

Additional Materials Required

- Purified RNA
- Dry bath with lid or a PCR thermal cycler.
- Pipettes with filtered, disposable tips
- Microcentrifuge
- 0.2 mL PCR tubes, strips, or plates with caps
- RNA purification kit to prepare RNA from collected samples. 10 µL of purified RNA in ultra-pure water is required per well of the RayBio® SARS-CoV-2 RNA Isothermal Amplification Colorimetric Assay Kit. The recommended minimum amount of RNA per well is 100 ng.
- Plate reader capable of UV/Vis OD measurement at 440 nm and 560 nm (optional)

V. Assay Protocol

A. Preparation of Reagents

- Thaw all reagents on ice prior to the assay.
- To prepare the positive standard RNA sample:
    1. Pipette 5 µL of the provided SARS-CoV-2 Synthetic Nucleocapsid RNA into a new tube.
    2. Dilute with 75 µL of Ultra-pure DEPC water.
    3. Mix well to generate 62.5 copies/µL of synthetic RNA ("Working Stock").
    4. Mix 10 µL of the "Working Stock" of synthetic RNA with 15 µL of the RayBio® SARS-CoV-2 RNA Isothermal Amplification Master Mix. The final concentration of this positive control is 25 copies/µL.

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.*

B. Assay Procedures

1. Obtain a 0.2 ml PCR tube for each sample to be run, including at least 1 additional well for a positive control, and 1 additional well for a negative control. *All reactions should be kept on ice during preparation.*

2. Prepare PCR Master Mix by adding the following components together (per sample or control):

    - 15 µl of RayBio® SARS-CoV-2 RNA Isothermal Amplification Master Mix
    - 10 µL of purified RNA in DEPC water

    Note: For a "negative control," add 10 uL DEPC water in lieu of RNA.
    Note: Analyzing samples in duplicate or triplicate is recommended.

3. 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 is not recommended as this can cause cross contamination between samples.

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

5. Put the samples in a heating block (i.e., dry bath) set to 60ºC or a PCR thermal cycler set at 60°C for 20 minutes.

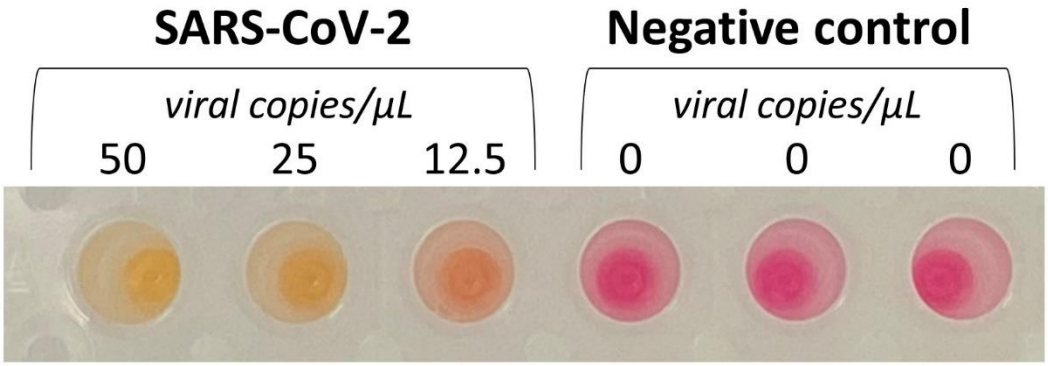
Note: Because RT-LAMP amplifies the DNA quickly, all samples with at least 25 copies/µL of the SARS-CoV-2 virus will eventually reach the saturated platform stage. Therefore, 20 minutes is the optimal incubation length to accurately assess the presence of viral RNA in the samples against the positive and negative controls. Deviations from this incubation length may result in data that is difficult to interpret.

6. Stop the reaction by transferring the tubes or plate to ice.

7. The presence of viral RNA in the sample can then be determined by eye or with a plate reader capable of measuring absorbance at 440 nm and 560 nm (see next section).

VI. Data Interpretation

The RT-LAMP colorimetric master mix contains a pink dye that turns to yellow as DNA is amplified. More DNA amplification will result in a more yellow color.



The sample color changes from pink to yellow when the SARS-CoV-2 cDNA is amplified

The results can be interpreted using the following methods:

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

   Note: A sample that has a yellow hue, but with an intensity less than the positive control should be considered as "suspect." It is recommended that the sample is run again with a higher total RNA content.

2. **With a plate reader:** Depending on your instrument, the reaction may need to be transferred to a new plate or tube and diluted. Measure the yellow and pink absorbances at 440 nm and 560 nm, respectively. Divide the sample optical density (OD) at 440 nm by the OD at 560 nm

(OD440 nm / OD560 nm). A ratio over 1.4 indicates that SARS-CoV-2 viral RNA is present (positive result), while a ratio less than 1.4 indicates that the samples does *not* have SARS-CoV-2 viral RNA (negative result).

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 ≥ 25 viral copies per µL in the reaction reached saturation.

Note: A sample having a yellow hue with an OD 440/560 nm ratio less than 1.4 should be considered as "suspect." It is recommended that the sample is run again with a higher total RNA content.

Correlation of RNA copies to OD 440/560 nm Ratio**

RNA copies / µL	OD 440/560 nm*
0	0.99
3.125	1.08
6.25	1.68
12.5	1.91
25.0	2.07
50.0	2.11
100.0	2.09
200.0	2.14

* 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
	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 allowed to run long enough	Allow the reaction to continue for additional time
	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.
	The reaction was not performed at the appropriate temperature	Check calibration of the instrument used to maintain temperature
	The reaction was allowed too occur for too long	Rerun the assay with a shorter run time

VIII. Note

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