Protocol

SARS-CoV-2 RT-LAMP kit

Vienna BioCenter, version January 2021

The SARS-CoV-2 RT-LAMP kit from the Vienna BioCenter is a tool for the detection of RNA (genetic material) of SARS-CoV-2, and is intended for research use only. These instructions will guide you through the use of the kit to successfully perform LAMP reactions with the supplied reagents on samples from the upper respiratory tract (swabs, gargle or saliva) taken in common collection media (HBSS, 0.9% saline or water (for gargle); UTM, 0.9% saline or HBSS (for swabs)).

Kit contents

RT-LAMP kit for 100 reactions (20 µl reaction volume)

Quantity	Number	Tube name	Contents
1x	1A	QE	QuickExtract - inactivation solution
1x	2A	LAMP mix	RT-LAMP mix
1x	3A	SARS-2 primer	primers for SARS-CoV-2 detection (*Orf1ab* gene)
1x	4A	HNB dye	hydroxynaphthol blue colorimetric dye
1x	5A	pos CTRL	non-infectious positive control
1x	6A	CTRL primer	human sample control primer mix (*ACTB* gene)
1x	7A	FLUO dye	fluorometric dye

IMPORTANT: Store the contents of the kit at -20 ℃. Before use, thaw reagents at room temperature and use immediately. Pay special attention to tube 2A LAMP mix - if a white precipitate is present, vortex or pipette up and down to dissolve the precipitate completely before starting assembling the reaction mix. Kit contents are stable for 6 months after shipment if stored at the recommended temperatures.

Materials required but not in the kit

- Disposable powder-free gloves and any additional PPE required
- 10 µl, 200 µl, and 1000 µl aerosol barrier tips
- Sterile, nuclease-free 1.5 ml microcentrifuge tubes
- 0.2 ml PCR tube strips with corresponding caps or 96-well PCR reaction plates with corresponding tight-sealing plate seals, plate seal roller
- Racks for 1.5 ml microcentrifuge tubes and 96-well 0.2 ml PCR reaction tubes
- Laboratory marker
- 10 µl, 100 µl and 1000 µl micropipettes
- 0.5 - 10 µl, 10 - 100 µl 8- or 12-channel pipettes (recommended for testing more samples)
- Reagent reservoirs (recommended for testing more samples)
- -20 ℃ freezer and 4 ℃ fridge for reagent storage
- Thermocycler, heat block or water bath that can be set to 95 °C
- Thermocycler, heat block or water bath that can be set to 63 °C
- Amplicon- and template-free workstation (UV lamp), BSL2 safety workstation with laminar flow HEPA filters, Vortex Mixer, Tabletop Microcentrifuge

Important

- SARS-CoV-2 is an infectious respiratory virus. When working with non-inactivated samples, do so only with appropriate safety equipment and in a laminar flow hood with a HEPA filter. If you are not sure of the proper safety procedures, check the information on working with SARS-CoV-2 from your local public health agency, like the ECDC or the CDC.

- This method is compatible with saliva, swabs collected in VTM, UTM or saline (0.9% NaCl), as well as gargle when water, HBSS or saline (0.9% NaCl) are used as gargling solutions. It is not compatible with virus inactivation swab media.

- Never open finished reaction tubes, as you risk contaminating your workspaces.

- Store the reagents at -20 ℃ to ensure their stability and thus the accuracy of your reactions.

- Running a positive and negative control with every testing session is crucial.

- If fluorescent detection is desired, add 0.4 µl of fluorescent dye (tube 7 - FLUO dye) into the reaction mix per sample. Run the reactions in a real-time thermocycler for 35 cycles at 63°C with 1-minute cycle length and reading at the end of each cycle. The fluorescent dye requires data acquisition using a standard FAM or SYBR filter (494nm/518nm absorption/emission).

- It is possible to use the tube nr. 6 - CTRL primer as a control for human material content. This primer initiates LAMP reactions in the presence of human beta actin transcript, thus you can check for the sample integrity of the given sample. For this use, prepare a second reaction mix in addition to the mastermix with SARS-CoV-2

primers by preparing the master mix according to the table but instead of primers from tube 3 add primers from tube 6. Test samples for both SARS-CoV-2 and human beta actin.

- Both PCR strips and 96-well plates must be properly sealed. This is absolutely crucial to prevent cross-well contamination. Plate seals (e.g. tight sealing plastic covers or aluminium seals), a plate-roller (e.g. BioRad) or even better a PCR plate sealer (e.g. BioRad PX1 PCR plate sealer) can help prevent cross-well contamination.

Protocol

1. Thaw tube number 1A (QE) and distribute 10 µl of the solution into a well of a PCR strip or 96-well plate according to the number of samples you intend to test.

2. While complying with the safety recommendations applicable for work with potential SARS-CoV-2 positive samples (e.g. S2 safety hood; PPE), add 10 µl of sample to the QuickExtract. Pipette up and down briefly to mix the sample and QuickExtract. Repeat for all samples.

3. Cap the PCR strip or seal the 96-well plate and incubate at 95 ℃ for 5 minutes. After 5 minutes, you can take out the now non-infectious inactivated samples from the incubator and work with them under standard laboratory conditions.

4. Prepare the RT-LAMP reaction mix according to the following table, multiplying the amount to add per one reaction by the number of samples being tested plus two (these two are for a positive and a negative control that should always be run alongside your test samples) with a 10 % excess to account for pipetting error. Work swiftly and work on ice if possible.

Reagent	Volume per one reaction
2A - LAMP mix	11 µl
3A - SARS-2 primer	2.5 µl
4A - HNB dye	2.5 µl

5. Dispense 16 µl of the reaction mix into PCR strips or a 96-well plate. Dispense enough master mix for each of your samples plus two reactions (+ and - control).

6. Add 4 µl of heat-inactivated QuickExtract + sample mixture to 16 µl of reaction mix, pipette up and down 10 times to mix the sample and reaction mix thoroughly. Repeat for each sample. In the same fashion, add 4 µl of positive control (tube 5) into the last well. Add 4 µl of negative control (not provided with the kit; this can be either water or a previously confirmed, inactivated negative sample) to the second to last well.
   Caution: Check that the color does not change when pipetting the samples into the reaction. The color should stay purple.

7. Cap the PCR strips or seal the 96-well plate. Take an image of the prepared reactions with a smartphone under good light conditions (to compare the color before and after incubation).

   Caution: If using a plate, take care of sealing the plate tightly using a roller!

8. Incubate the reactions at 63 ℃ for 35 minutes on a stable heat source such as an incubator or a thermocycler. Do not exceed the stated incubation time.

9. After 35 minutes, take out the PCR strips or 96-well plate and immediately inspect the reactions visually. Take an image of the reactions under good light conditions. Positive reactions are sky blue, while negative reactions stay purple. For easier classification, you can upload your image to the web app colorimetry.net which enhances the color difference. Always compare the color of the sample to colors of the positive and negative controls'. Discard reactions at once.
   Caution: Never open the reactions. Opening the reactions can lead to severe contamination.

Troubleshooting

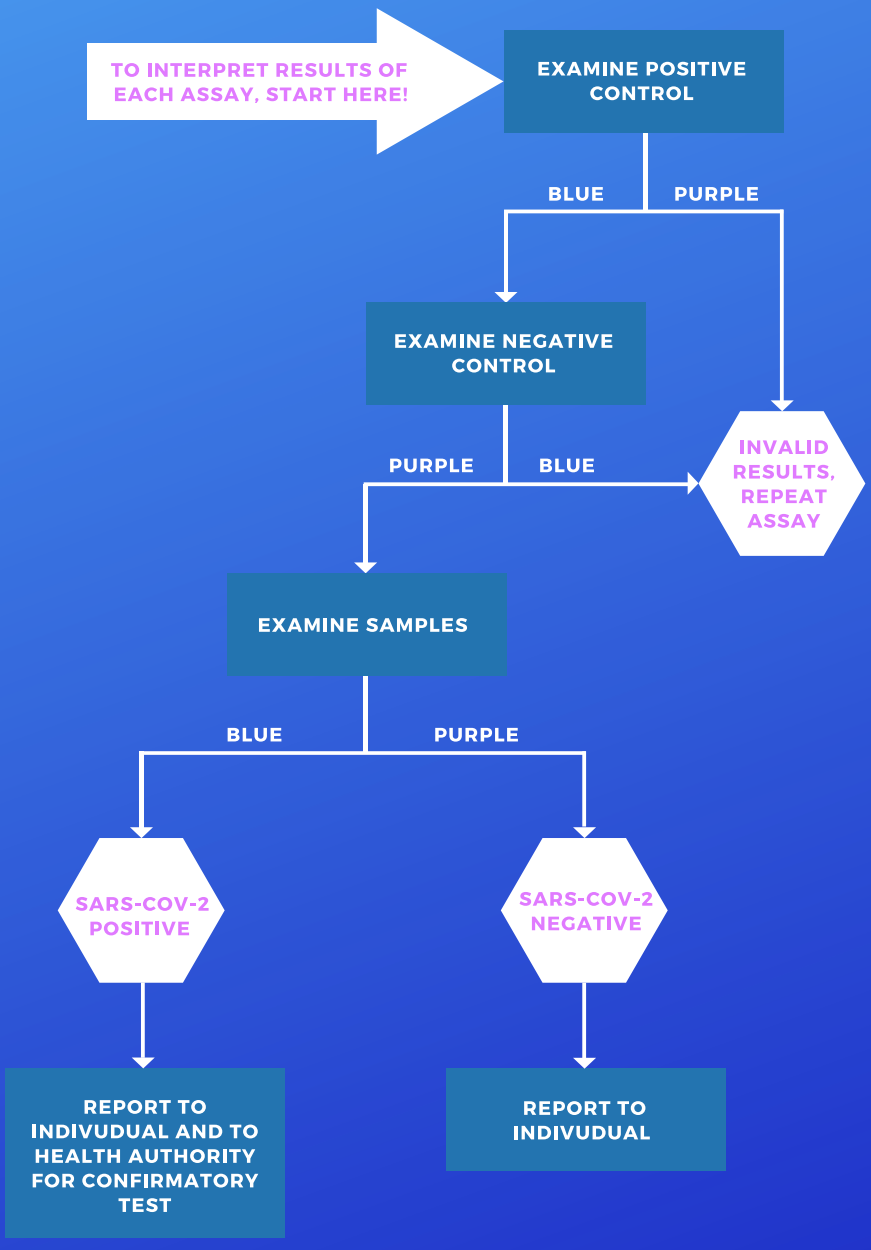
Problem: The color of my RT-LAMP reaction changed upon the addition of the sample even before incubating it.
Solution: What has likely happened is the sample collection medium contained some chemical ingredient that inhibited the color readout with HNB. This is most often either a chaotropic agent such as guanidium or a metal chelator such as EDTA. This sample is then incompatible with RT-LAMP.

Problem: I find it very hard to see the color differences between positive and negative reactions.
Solution: For making the color differences more noticeable to the human eye and to enable color blind users to evaluate the reactions, you can use the simple web app colorimetry.net where you can upload your image and the site provides you with a color stretched image, where the color differences are amplified, and a color switched image, which switched the color by 180 degrees in the color space.

Problem: I have a sample that has changed color slightly, but not as strongly as a full positive.
Solution: Retest the sample, in duplicates if possible. Consider contacting the health authorities for a diagnostic test.

RT-LAMP
evaluation flowchart

without sample quality control



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