Protocol

SARS-CoV-2 bead-LAMP kit

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The SARS-CoV-2 bead-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. Bead-LAMP is a sensitive, isothermal nucleic acid amplification method utilizing a bead-enrichment step coupled with a reverse transcribed loop-mediated isothermal amplification reaction. It delivers a precise, colorimetric yes/no result for the presence of SARS-CoV-2 nucleic acids in a crude sample within 45 minutes.

These instructions will guide you through the use of the kit to successfully perform bead-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

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

Quantity	Number	Tube name	Contents
1x	1B	QE	QuickExtract - inactivation solution
1x	2B	bLAMP mix	bead-LAMP mix
1x	3B	SARS-2 primer	primers for SARS-CoV-2 detection (*Orf1ab* gene)
1x	4B	GuHCl	guanidine hydrochloride solution
1x	5B	pos CTRL	non-infectious positive control
1x	6B	mag BEADS	magnetic beads

IMPORTANT: Store all reagents except tube 6B at -20 ℃. Store tube 6B at 2 to 8 ℃. Before use, thaw reagents at room temperature and use immediately. Pay special attention to the bLAMP mix - if a white precipitate is present, vortex or pipette up and down to dissolve the precipitate completely. Do not transport the kit on dry ice, as that can influence the chemistry of the bLAMP mix and lead to indeterminate results. 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
- 85 % ethanol (molecular biology grade, dilute with nuclease-free water)
- Magnetic separation stand for 8-well PCR strips or 96-well plates
- 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 and 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 the stated temperatures to ensure their stability and thus the accuracy of your reactions.

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

- 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 contamination.

Protocol

1. Prior to performing bead-LAMP, take out the tube nr. 6B (mag BEADS) from the fridge and leave at room temperature for about 30 minutes to let the mixture warm up. Mix the magnetic bead solution thoroughly by vortexing or pipetting up and down until it is fully homogeneous.

2. Thaw tube number 1B (QE) and pipette 50 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.

3. While complying with the safety recommendations applicable for work with potential SARS-CoV-2 positive samples (e.g. BSL2 safety hood; PPE), add 50 µl of sample to the QuickExtract. Pipette up and down briefly to mix the sample and QuickExtract. Repeat for all samples.

4. 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.

5. Prepare the bead-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
2B - bLAMP mix	10 µl
3B - SARS-2 primer	5 µl
4B - GuHCl	5 µl

6. Briefly spin down the inactivated samples to collect all liquid from the lids and walls to the bottom of the wells. To 100 µl of inactivated sample, add 60 µl of magnetic beads from tube nr. 6B (mag BEADS). Pipette up and down to mix the viscous bead mixture with the sample. Repeat for all samples. The use of a multichannel pipette is recommended.

7. Incubate the bead-sample mixture at room temperature for 5 minutes. This lets the RNA bind to the beads.

8. After 5 minutes, place the samples onto a magnetic separation stand for 5 minutes to allow for complete separation of magnetic particles from the rest of the solution.

9. While keeping the samples on the magnetic stand, remove all of the solution, taking care not to disturb the beads in the process.

10. While keeping the tubes on the magnetic stand, add 200 µl of 85% EtOH solution to the beads and wait for 30 seconds, then remove the wash solution. Work swiftly and make sure to take out all visible ethanol from the tubes.

11. Dry the beads briefly by leaving them at room temperature for 3 minutes. Make sure not to over-dry the beads, as leaving them out for longer than 5 minutes can make them look coarse, dried and light colored, which has a negative impact on sample recovery and sensitivity of the whole assay.

12. Immediately distribute 20 µl of bead-LAMP reaction mix prepared in step 5 directly to the beads in the sample wells. Resuspend the beads in the solution by pipetting up and down or capping the PCR strips/sealing the 96-well plate and carefully vortexing.

13. Cap the PCR strips or seal the 96-well plate if you haven't done so in step 12. 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!

14. Incubate the reactions at 63 ℃ for 25 minutes on a stable heat source such as an incubator or a thermocycler. Do not exceed the stated incubation time.

15. After 25 minutes, take out the PCR strips or 96-well plate and inspect the reactions visually. Take an image of the reactions under good light conditions. Positive reactions are yellow, while negative reactions stay pink/red. Always compare the color of the sample to the positive and negative control's color. Discard reactions immediately.
    Caution: Never open the reactions. Opening the reactions can lead to severe contamination.

Troubleshooting

Problem: My sample turned yellow before I even put it in the incubator / thermocycler / water bath, what is happening? And can I continue?
Solution: A positive result can never be produced before incubating the sample at 63°C, this is most likely a result of leftover ethanol being present in the wells before the addition of the bead-LAMP reaction mix. Make sure to remove all the ethanol you can from the wells by pipetting it out, and letting the beads dry for 3 - 5 minutes at room temperature. When you see a color change in one well before incubation, this sample will be inconclusive, and you have to start over to get relevant results.

Problem: The color of my bead-LAMP reaction is orange, somewhere in the middle between yellow and pink. How do I interpret this?
Solution: For indeterminate samples, we recommend repeating the reaction. Whenever you are not sure, compare the sample color to the color of a positive control. This is why we recommend everyone to run a positive and a negative control in every test.

Problem: I want to use bead-LAMP to pool my samples and cut down on the number of reactions. Can I pool these samples before inactivation to save on the amount of

inactivation solution I use?
Solution: Yes, pooling samples before inactivation is just as valid as pooling them after inactivation. When pooling before inactivation, one has to adhere to proper protective precautions as with infectious sample handling. This approach helps you save money on the inactivation reagent used, but in case of a positive pool you will have to go back and inactivate the individual samples and re-test them, to find the positive sample from the pool.

Problem: How many samples can I pool at once?
Solution: That depends on many factors, such as the sensitivity you are aiming to achieve and the percentage of positives in your sample set. To make an educated decision, we recommend reading up on pooling strategies for SARS-CoV-2 testing. Pooling strategies for PCR are applicable for bead-LAMP. In general we recommend pooling 10 samples.

bead-LAMP
evaluation flowchart