Product information
Applyo RT-LAMP SARS-CoV-2 Beads (RUO)

Abstract

Applyo RT-LAMP SARS-CoV-2 Beads (RUO) are lyophilized master mixes containing all essential reagents required for performing a LAMP-assay for detection of SARS-CoV-2-RNA. Only the template has to be added. The beads are premixed, designed for a single reaction, ready-to-use and instantly resuspending.
The LoD of Applyo RT-LAMP SARS-CoV-2 Beads was verified for SARS-CoV-2 detection of N2 and E genes to ≥ 100 cp/reaction at 25 µL reaction volume using digital-PCR quantified RNA (EURM-019 [REF 1]). The shelf-life of the product is 1 year when stored at room temperature (T ≤ 21°C).

Contents

Loop-meditated amplification principle

Loop-mediated amplification (LAMP) is an isothermal amplification mechanism leading to long concatemers of the target sequence.

In the first step of the RT-LAMP reaction, viral RNA is transcribed into cDNA (**FIG. 1A**) primed from primer B2.

Subsequently, the target sequence is amplified utilizing 6 primers per target (**FIG. 1B ff.**). Both, an inner (F2/B2) and an outer primer (F3/B3) can hybridize to the cDNA template. Inner primers F2/B2 carry a 5' sequence complementary to F1/B1. Once the inner primers are elongated, the outer primers hybridize and the DNA polymerase displaces the inner primer with a strong strand displacement activity (**FIG. 1C**), allowing the complementary regions F1/F1c & B1/B1c to hybridize (**FIG. 1D**). Upon hybridization on both sides of the product (**FIG. 1E+F**) the LAMP-typical "dumbbell structure" is formed (**FIG. 1G**). Now primers complementary to sequences F2/F2c/B2/B2c can hybridize (**FIG. 1H**) and prime elongation leading to large concatemers (**FIG. 1I**) of the desired sequence, which can be detected subsequently.

Product description

Applyo RT-LAMP SARS-CoV-2 Beads contain all reagents in a lyophilized bead format, required for a dual colorimetric & fluorescent isothermal RT-LAMP reaction to detect SARS-CoV-2. A reverse transcriptase for cDNA transcription as well as a strand displacing DNA polymerase and all LAMP-primers are included. Only the addition of template is required.

The reactions are simultaneously suitable for two independent detection technologies – a visual colorimetric (red – green) and a fluorescence (488 nm FAM-channel) read out in any compatible device. The beads are designed for a single reaction, pre-mixed with all required reagents, ready-to-use and instantly resuspending once the sample is added.

The starting point of the RT LAMP-reaction is well defined as both enzymes carry a hot-start function, which is activated at temperatures above 60°C.

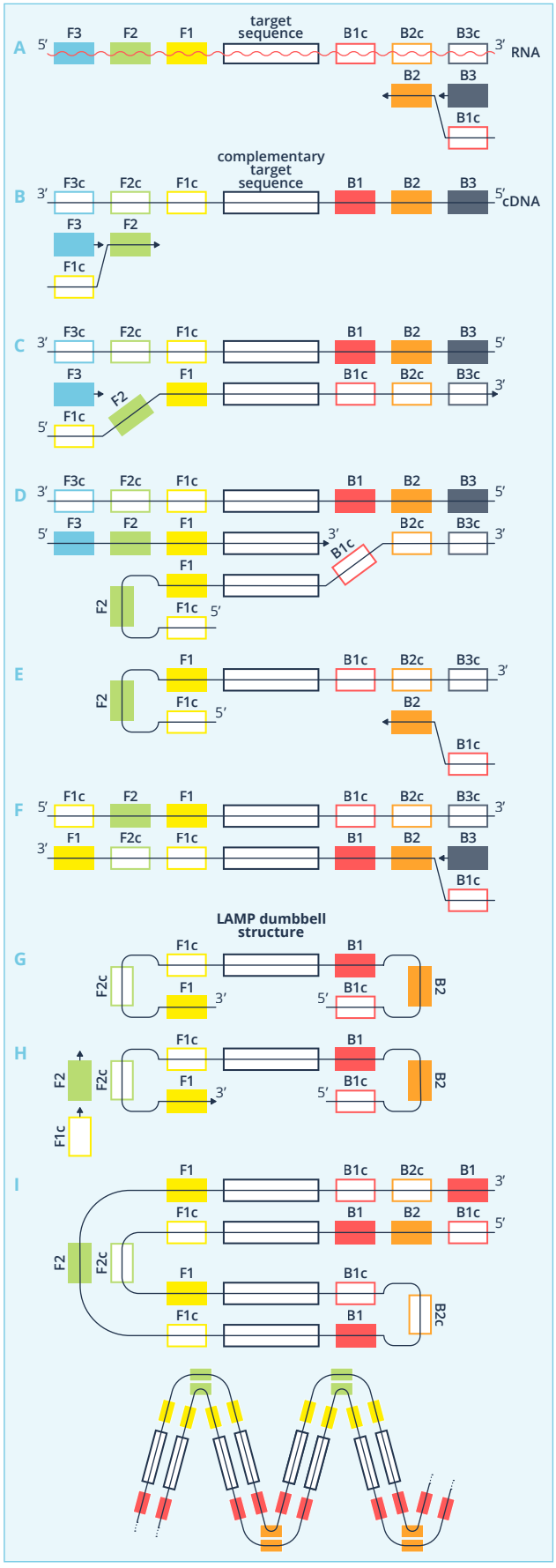


Fig. 1 Schematic illustration of SARS-CoV-2 RNA viral genome
Wavy line – RNA, straight line – cDNA, framed rectangles are complementary sequences of equal color filled rectangles

The Applyo RT-LAMP SARS-CoV-2 Bead is a dual target assay ensuring highly sensitive detection of SARS-CoV-2 as both, E- and N2-gene from the SARS-CoV-2 viral genome are detected simultaneously (**FIG. 2**).

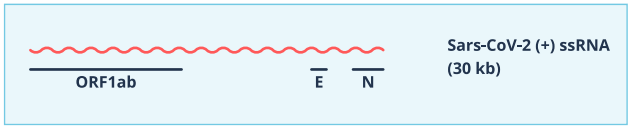


Fig. 2 Loop-mediated amplification principle

Each Applyo Bead is designed for a single RT-LAMP SARS-CoV-2-reaction-well. Hence, errors in assembling the RT-LAMP SARS-CoV-2-reaction are circumvented and pipetting efforts reduced substantially. With this convenient format the risk of cross-contamination during handling is considerably reduced.

Due to the special freeze-dried formulation of the Applyo Beads, the reagents are easy to handle and long-term storage stable at ≤ 21°C. It is not necessary to place the Applyo Beads on ice during handling. Just reconstitute the beads in a suitable aqueous solutions containing the sample at room temperature (see below for further details).

Applyo RT-LAMP SARS-CoV-2-beads are optimized for isothermal detection of ≥ 100 cp/reaction in a LAMP-reaction.

Reagents provided

8 ready to use Applyo RT-LAMP SARS-CoV-2 Beads individually packed in low profile PCR-tubes. Each Applyo Bead contains:

- DNA-polymerase with strand displacement activity
- Reverse transcriptase
- dNTPs consisting of an equimolar mixture of dATP, dCTP, dGTP and dTTP
- LAMP-primers for E-gene
- LAMP-primers for N2 gene
- LAMP-buffer components
- Colorimetric dye
- Fluorescent dye

for one 25 µl RT-LAMP SARS-CoV-2-reaction.

Reagents & Materials required but not provided

- Custom RNA template to be amplified
- PCR-clean water
- Pipettes
- PCR-clean pipette tips (preferable filter tips to prevent carry over)
- Small centrifuge to spin down reaction-tubes
- Thermal heater or Real-Time PCR-cycler

Handling of Applyo Beads

Applyo Beads are robust under ambient conditions. Exposure to high moisture (> 80 % rH) over 30 minutes or placing Beads on ice could result in moisture condensation and may impair the ability of the Applyo Beads to solve properly and thereby impact LAMP-performance. Therefore, limit the exposure to humidity (e.g. in the air) to a minimum until the bead is reconstituted with the primer-probe mixture.

Remove the required amount of tubes with Applyo Beads from the package and make sure to carefully reseal the package to protect the remaining Applyo Beads from moisture from the environment. Resealed Applyo Beads should be used within 3 months.

Open the tube just right before addition of buffer.

Solve the Applyo Bead directly in the tube that it is provided in.

To resuspend the Applyo Bead, close the tube and flick the tube 5x. Spin down afterwards and store in standard amplification applications at cold conditions before heating to reaction temperature.

Sample preparation

Note that RNA is sensitive to nucleases, therefore handle RNA samples on ice.

Prepare the RNA-sample as usual and provide the sample in PCR-clean water or 10 mM Tris pH 8.0-buffer. The RNA sample can contain a buffer, but $MgCl_2$ should be avoided in the sample as it may interfere with the assay.

Add up to 24 µl of the sample containing ≥ 100 copies per reaction to resuspend the Applyo Beads.

Tab. 1 Addition of template to Applyo Bead

Number of reactions	1
Bead	1 µl*
Template + PCR-grade water	in total 24 µl
Final reaction volume	25 µl

* Be aware that solving the bead will add approximately 1 µl of volume to the total reaction volume.

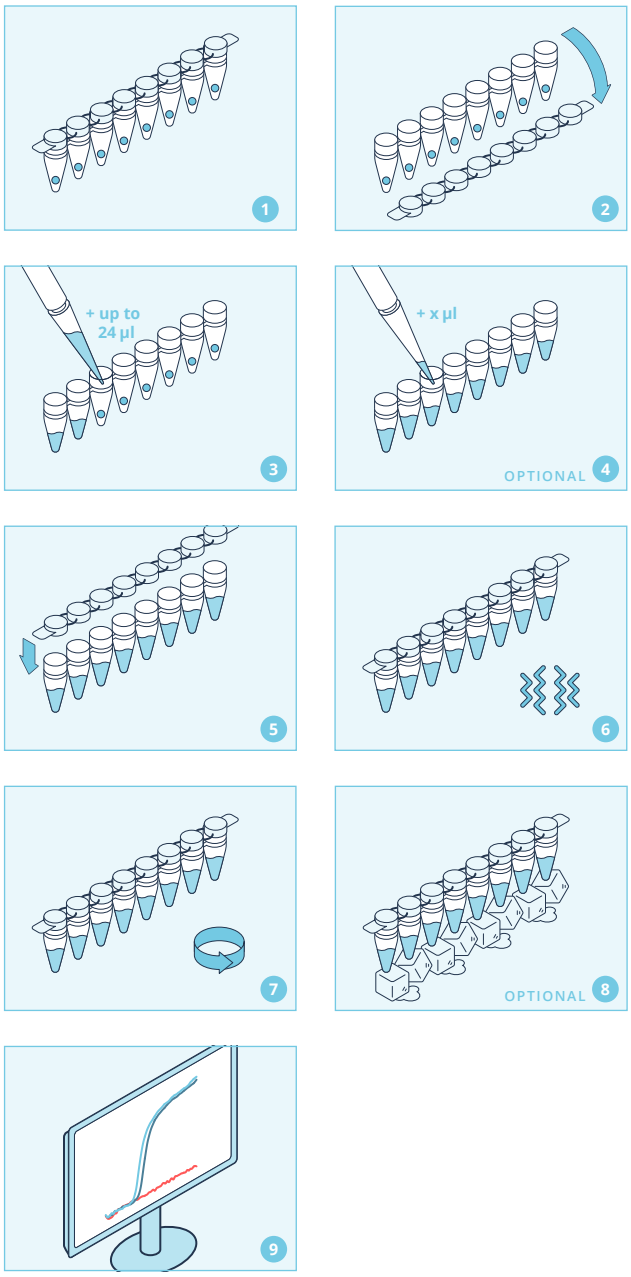
Procedure

- Prepare the template RNA (see “Sample preparation” for details).

1. Take desired number of tubes with Applyo Beads from the packaging pouch and place them with the seal still intact in rack at room temperature. Carefully reseal the package to protect the remaining Applyo Beads from moisture from the environment.
2. Open the tubes with Applyo Beads.
3. Reconstitute the Applyo Beads with 24 µl of sample (+ PCR-grade water, if required, see above).
4. **OPTIONAL**
   If RNA-sample and PCR-grade water are added in two steps, add the higher volume first.
5. Seal plate for RT-LAMP SARS-CoV-2-Reaction.
6. Mix
7. Spin down

- Place plate, stripe or tubes in cycler or thermal heater.

8. **OPTIONAL**
   Store tubes on ice for later processing.
9. Run RT-LAMP SARS-CoV-2-reaction at 65°C for 30 min
9. Readout results (fluorescence; intensity, see **FIG. 3**) or by eye (colorimetric – color change red = negative to green = positive, see **FIG. 4**).



Storage of Beads

Store at ≤ 21°C. Short term storage at temperatures up to 30°C is also possible.

Precautions and disclaimer

This product is for R&D use only, do not use for diagnostic, prevention or treatment of disease purposes. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

References

[1] https://crm.jrc.ec.europa.eu/p/EURM-019

Application specialist

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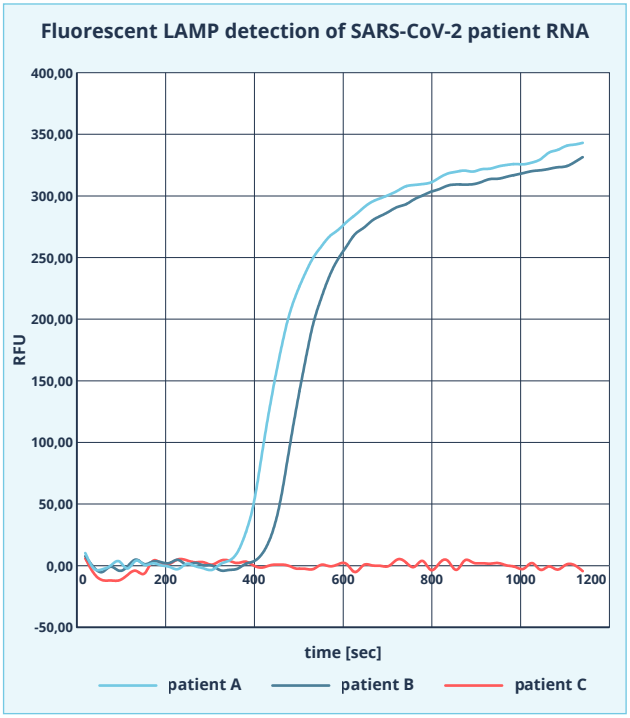

Fig. 3 Fluorescence readout over time: Patient RNA-samples: blue – positive detection, red – negative signal

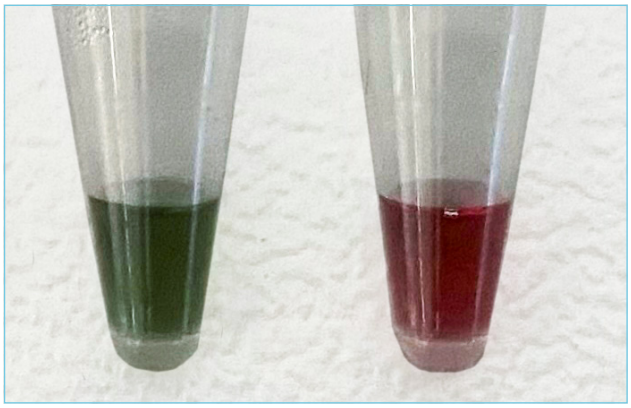

Fig. 4 Colorimetric detection of LAMP-assay; green = positive detection; red = negative signal for SARS-CoV-2