

Applyo dual-detection RT-LAMP Beads

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1. General handling of Applyo Beads

Applyo dual-detection RT-LAMP Beads are ready-to-use, lyophilized reagent mixtures containing all necessary components to perform a generic LAMP reaction. The Beads contain a fluorescent dye to monitor template generation in real-time as well as a colorimetric detection system, which shows a positive amplification by a red → green colour change. For an instantly resuspending only aqueous solutions of custom specific primers and template have to be added.

Applyo Beads are robust for storage and application under ambient temperature. Exposure to high moisture (> 60 % rH) for 30 minutes or placing Applyo Beads on ice (due by condensed moisture) may impair the rapid solubility of the Applyo Beads. Therefore, limit the exposure to humidity until resuspension.

2. Preparation

Check that all reagents and materials, which are required but not provided are available:

- Custom primers and probes at preferably 10 µM stock concentration
- Custom RNA template to be amplified in PCR-grade water or 10 mM Tris-HCl pH 8.0
- PCR-clean water
- Pipettes
- PCR-clean pipette tips (preferable filter tips to prevent carry over)
- Small centrifuge to spin down PCR-tubes
- Vortex or other means to mix reagents
- Isothermal fluorescent/colorimetric reader or thermal incubator or thermal cycler

3. Protocol

Applyo dual-detection LAMP Beads are optimized for amplification of RNA-molecules with a length of 70–150 bp. The following LAMP-protocol is suitable for many standard templates; however, individual custom templates might require optimization of the PCR-protocol.

- Prepare custom primers and probes as 100 µM stock solution*
- Prepare custom primer-master mix according to **TAB. 1** for n+1 reactions*. It is recommended to prepare a larger master mixes of the primers which can be used in multiple experiments:

TAB. 1 Prepare custom specific primer-probe-mix

Number of reactions	1	8 (+1)	Final Concentration in LAMP*
100 µM Primer FIP / F2-F1c*	0.4 µl*	3.6 µl	1.6 µM
100 µM Primer BIP / B2-B1c*	0.4 µl*	3.6 µl	1.6 µM
100 µM Primer F3*	0.05 µl*	0.45 µl	0.2 µM
100 µM Primer B3*	0.05 µl*	0.45 µl	0.2 µM
100 µM Primer LOOP F*	0.1 µl*	0.9 µl	0.4 µM
100 µM Primer LOOP B*	0.1 µl*	0.9 µl	0.4 µM
PCR-clean Water	7.9 µl*	71,1 µl	–
TOTAL	9 µl*	81 µl	–

* Please adjust according to custom specific protocol

- Prepare RNA template as usual and provide in PCR-grade water or 10 mM Tris-HCl pH 8.0 to a final concentration of 1.3–66,666 cp/µl (e.g. 20–1,000,000 cp in 15 µl sample volume)

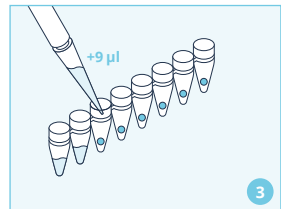
TAB. 2 Addition of template to bead-primer-mix

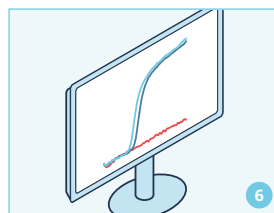
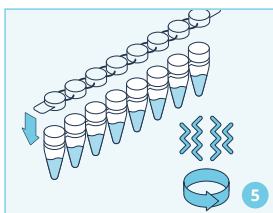
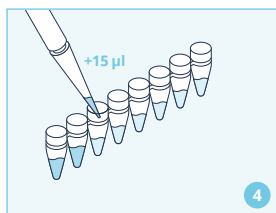
Number of reactions	1
Primers*	9 µl*
Bead(s)	1 pc#
Template	15 µl*
Final volume	25 µl

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

* Please adjust according to custom specific protocol.

- 1 Take desired number of tubes with Applyo Beads from the resealable packaging pouch and place them with the lid still intact in rack at room temperature. Carefully reseal the package to protect the remaining Applyo Beads from environmental moisture.
- 2 Open the tubes with Applyo Beads right before addition of primer-probe mix.
- 3 Reconstitute the Applyo Beads with 9 µl of primer-probe mix.





- 4 Add 15 µl template to each tube
- 5 Seal tubes for PCR, Mix, Spin down
- 6 Place tubes in Isothermal fluorescent/colorimetric reader or thermal incubator or thermal cycler
OPTIONAL: Store tubes on ice for later processing
ATTENTION: When only one tube or stripe is applied, check that the isothermal fluorescent/colorimetric reader or thermal incubator used is compatible or whether empty tubes have to be placed elsewhere in the machine to prevent tube damage
- 6 Run LAMP at 65°C for 30 min, if fluorescent readout is desired, detect ~ every 30 sec.

Observe and evaluate the typical colour shift and the fluorescence signal redout. For example images see Ref 1.

4. Storage

Store at 20°C. Short term storage at temperatures ≤ 30°C is also possible.

5. 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.

6. References

[1] www.applyo-jena.com/wp-content/uploads/2023/03/Applyo-Jena-PI-RT-Lamp-SARSCoV2-Beads.pdf

7. Application Specialist

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