Product information
Applyo RT-qPCR Beads

Abstract

Applyo RT-qPCR Beads are lyophilized Mastermixes containing all essential reagents required for performing a generic single- or multiplex RT-qPCR assay. Only template specific primers, probes and the templates have to be added. The Beads are pre-mixed, designed for a single reaction, ready-to-use and instantly resuspending. The LoD of Applyo RT-qPCR Beads was verified for SARS-CoV2 detection of N1, E and RdRP genes to ≥ 20 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

Product description

Applyo RT-qPCR Beads contain all reagents (including polymerase and reverse transcriptase) required for a RT-qPCR reaction in a lyophilized bead format. Only primers and probes for user specific templates are required. The Beads are premixed, designed for a single reaction, ready-to-use and instantly resuspending reaction mixes.

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. During handling it is not necessary to place the Applyo Beads on ice. Just resuspend the Beads in a suitable aqueous solution at room temperature.

Each Applyo Bead is designed for a single RT-qPCR-reaction-well. Hence, errors in assembling the PCR-reaction are circumvented and pipetting efforts reduced substantially. With this easy-to-use format the risk of cross-contamination during handling is substantially reduced.

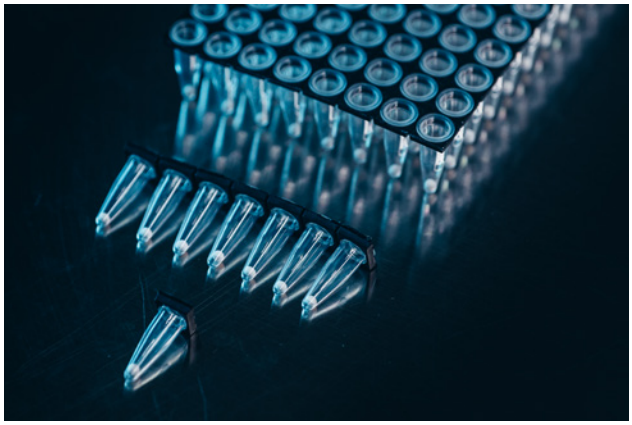


Fig. 1 Applyo RT-qPCR Beads are available as single tubes, 8-PCR-stripes or 96-well plate format

A M-MLV Reverse Transcriptase mutant expressed in *E.coli* is used in the first step of the RT-qPCR to translate template RNA into cDNA. The reverse transcriptase applied in the Applyo RT-qPCR Bead is highly thermostable up to 60°C, thereby allowing even difficult secondary-structured RNA templates to be transcribed. Prior to qPCR-amplification the RT is heat-inactivated and thereby loses its functionality in the subsequent amplification steps of the PCR-reaction.

The Taq polymerase is derived from *Thermus aquaticus MB* and expressed in *E.coli*. The hot-start is accomplished by reversible aptamer-binding which impairs the Taqs polymerase activity during assay preparation and the first RT-step at 50°C temperature but is released in the subsequent steps at temperatures > 55°C. Above 60°C the

enzyme is fully active. Due to its thermostable properties, the Taq-polymerase can run through repeated PCR cycles without significant loss of activity. The Taq-polymerase is compatible to Taq-Man probe detection and exhibits no detectable 3'→5' exonuclease activity.

All reagents utilized in the Applyo RT-qPCR-Bead are free from DNAse and RNAse.

In the course of the experiment customized primers and probes as well as the sample are added in liquid form to reconstitute the bead.

Applyo RT-qPCR Beads are optimized for detection of RNA-molecules with a length of 100 bp–500 bp and 20–100.000 copies/reaction for single and multiplex template detection.

Reagents provided

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

- Taq-DNA-polymerase
- Reverse transcriptase
- dNTPs consisting of an equimolar mixture of dATP, dCTP, dGTP and dTTP.
- Mg-salt
- PCR-buffer components

for one 25 µl PCR-reaction.

Reagents & Materials required but not provided

- Custom primers and probes at preferably 10 µM stock concentration
- Custom RNA to be amplified
- PCR-clean water
- Pipettes
- PCR-clean pipette tips (preferable filter tips to prevent carry over)
- Small centrifuge to spin down PCR-tubes
- Thermal 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 may result in moisture condensation and may impair the ability of the Applyo Beads to solve properly and thereby impact PCR-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 take care 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 bead directly in the tube that it is provided in.

To resuspend the bead, close the tube and flick the tube 5x. Spin down afterwards and store as in standard PCR applications at cold conditions before cycling.

Sample preparation

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

Prepare a 10 µM solution of the template specific primers and probes in nuclease-free water. Add 20 µl of this solution to the Applyo Bead before adding the sample.

Tab. 1 Prepare custom specific primer-probe-mix

Number of reactions	1	8 (+1)	Final Concentration*
10 µM Primer 1	1,5 µl	13,5 µl	600 nM
10 µM Primer 2	1,5 µl	13,5 µl	600 nM
10 µM Probe	0,5 µl	4,5 µl	200 nM
Nuclease-Free Water	16,5 µl	148,5 µl	

* Please adjust according to custom specific protocol

Prepare the RNA-sample as usual and provide the sample in PCR-clean water ddH_2O or 10 mM Tris-HCl buffer, pH 8.0.

Add 4 µl of the sample to the resuspended Applyo Beads containing 20–1,000,000 copies per reaction.

Tab. 2 Addition of template to bead-primer-probe-mix

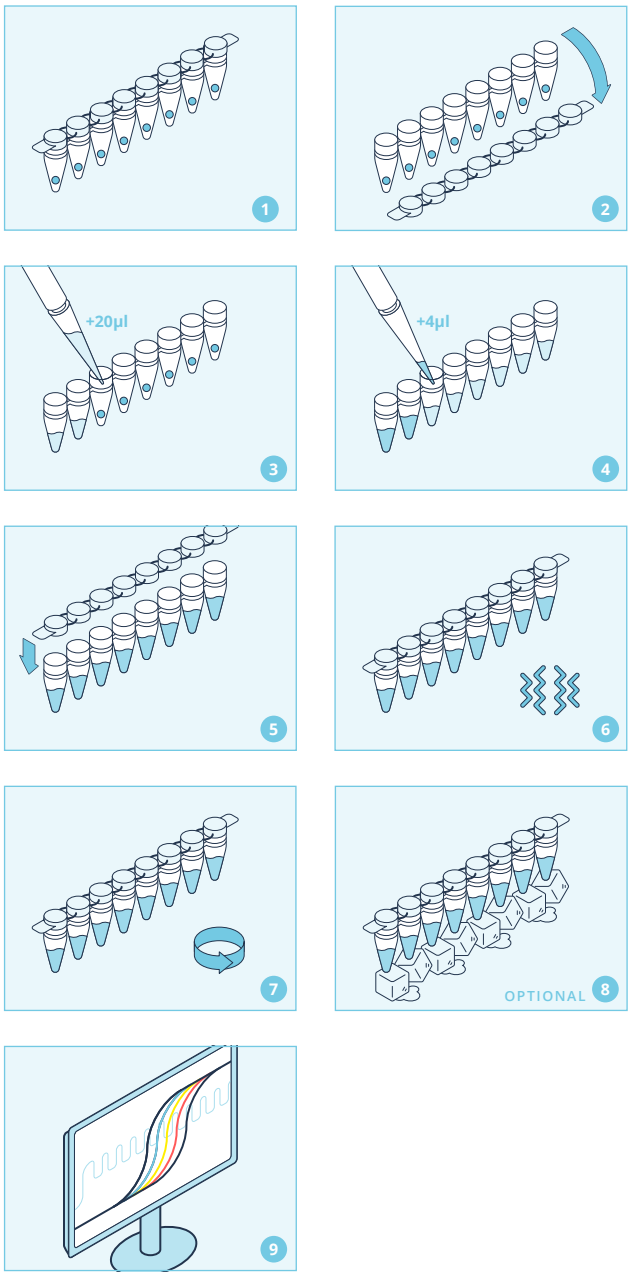
Number of reactions	1
Bead + primers + probe	21 µl*
Template	4 µl
Final volume	25 µl

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

Procedure

Applyo RT-qPCR Beads are optimized for amplification of RNA-molecules with a length of 100–500 bp. The following PCR-protocol is suitable for many standard templates, however, individual custom templates might require optimization of the PCR-protocol.

- Prepare PCR-primer-and-probe-mix according to **Tab. 1**. Prepare enough volume for the amount of desired PCR-reactions +1.
- Prepare the template according to **Tab. 2**.
- 1 Take desired number of tubes with Applyo Beads from the packaging pouch and place them with the seal still intact in a 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 (right before addition of primer-probe mixture)
- 3 Reconstitute the Applyo Beads with 20 µl of primer-probe mix.
- 4 Add 4 µl template to each tube
- 5 Seal plate for PCR
- 6 Mix
- 7 Spin down
- 8 **OPTIONAL**
  Store tubes on ice for later processing
- 9 Run PCR
  Adapt all T, t, and # of cycles to your assay specific t_m and properties.



Tab. 3 PCR-program; adjust according to custom specific protocol

Temperature*	Time*	
50°C	10 min.	
94°C	1 min.	
94°C	10 sec.	repeat 45x*
58°C	10 sec.	
75°C	20 sec.	
Signal readout		

* Please adjust according to custom specific protocol

Storage of Beads

Store at 20°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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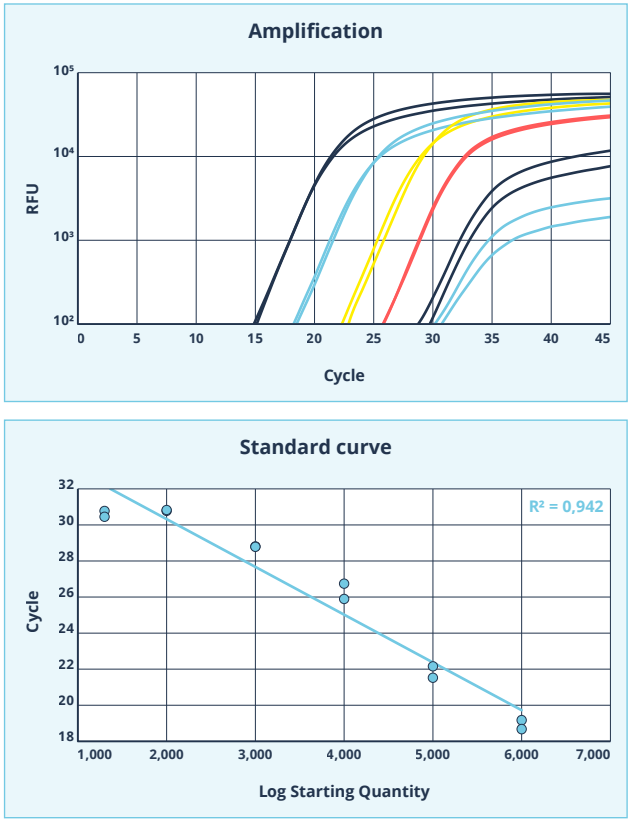

Fig. 2 Example data for application of Applyo RT-qPCR Beads: Quantification of RdRP-gene from SARS-CoV-2. Experiment was performed as described under procedure.