

# Applyo RT-qPCR Beads single reaction

**Contents**

|                                          |   |
|------------------------------------------|---|
| 1. General handling of Applyo Beads..... | 2 |
| 2. Preparation .....                     | 2 |
| 3. Protocol.....                         | 2 |
| 4. Storage.....                          | 4 |
| 5. Precautions and disclaimer .....      | 4 |
| 6. References.....                       | 4 |
| 7. Application Specialist .....          | 4 |

### 1. General handling of Applyo Beads

Applyo RT-qPCR Beads for probe detection are ready-to-use, lyophilized reagent mixtures containing all necessary components to perform a RT-qPCR reaction. Only aqueous solutions of custom specific primers, probes and template have to be added for an instantly resuspending.

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
- Thermal or Real-Time PCR-cycler

### 3. Protocol

Applyo RT-qPCR Beads are optimized for amplification of RNA-molecules with a length of 70–500 bp. The following PCR-protocol is suitable for many standard templates as well as intercalating dyes; however, individual custom templates might require optimization of the PCR-protocol.

- Prepare custom primers and probes as 10 µM stock solution\*
- Prepare custom primer-probe-master mix according to **TAB. 1** for n+1 reactions\*:

**TAB. 1** Prepare custom specific primer-probe-mix

| Number of reactions | 1       | 8 (+1)  | Final Concentration in PCR* |
|---------------------|---------|---------|-----------------------------|
| 10 µM Primer 1*     | 1 µl*   | 9 µl    | 400 nM                      |
| 10 µM Primer 2*     | 1 µl*   | 9 µl    | 400 nM                      |
| 10 µM Probe 1*      | 0,5 µl* | 4,5 µl  | 200 nM                      |
| PCR-clean Water     | 6,5 µl* | 58,5 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)

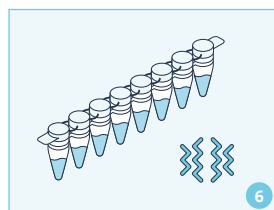
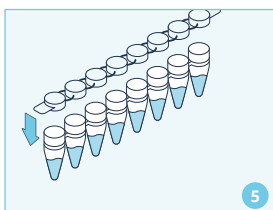
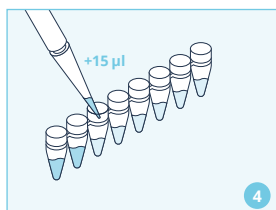
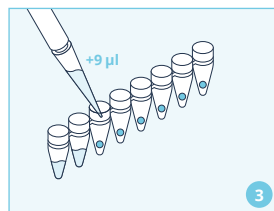
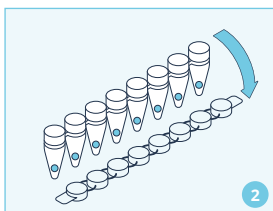
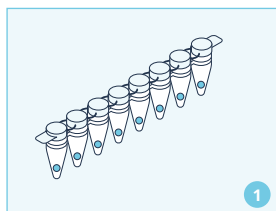
**TAB. 2** Addition of template to bead-primer-probe-mix

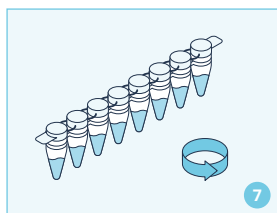
| Number of reactions | 1                 |
|---------------------|-------------------|
| Primers + probe*    | 9 μl*             |
| Bead(s)             | 1 pc <sup>#</sup> |
| Template            | 15 μl*            |
| Final volume        | 25 μl             |

<sup>#</sup> Be aware that solving the bead will add approx. 1 μl of volume to the total reaction volume.

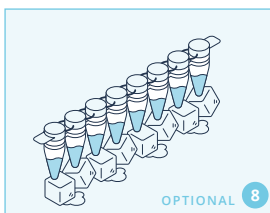
\* Please adjust according to custom specific protocol.

- 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 mixture.
- 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
- 6 Mix





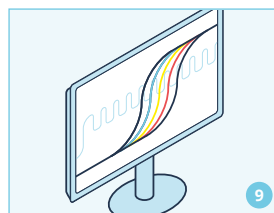
7 Spin down



8 Place tubes in PCR machine

**OPTIONAL:** Store tubes on ice for later processing

**ATTENTION:** When only one tube or stripe is applied, check that the PCR-machine used is compatible or whether empty tubes have to be placed elsewhere in the machine to prevent tube damage



9 Run RT-qPCR: Adapt all T, t, and # of cycles to custom assay specific tm and properties.

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

| Temperature*   | Time*               |
|----------------|---------------------|
| 50°C           | 3 min.              |
| 95°C           | 20 sec              |
| 95°C           | 1 sec.              |
| 58°C           | 20 sec. repeat 45x* |
| Signal readout |                     |

\* Please adjust according to custom specific protocol.

## 4. Storage

Store at 20°C. Short term storage at temperatures  $\leq 30^{\circ}\text{C}$  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/2022/11/Applyo-jena-PI-RT-qPCR-Beads.pdf](http://www.applyo-jena.com/wp-content/uploads/2022/11/Applyo-jena-PI-RT-qPCR-Beads.pdf)

## 7. Application Specialist

Applyo Jena GmbH

E-Mail: [info@applyo-jena.com](mailto:info@applyo-jena.com), Phone: +49-3641-5547880