

Applyo qPCR Bead 25x MM for probe detection

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1. General handling of Applyo Beads

Applyo qPCR Beads for probe detection are ready-to-use, lyophilized reagent mixtures containing all necessary components to perform a 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 to 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 DNA 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 qPCR Beads are optimized for amplification of DNA-molecules with a length of 70–500 bp. The following PCR-protocol is suitable for many standard templates; 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	2,5 µl*	22,5 µl	–
TOTAL	5 µl*	45 µl	–

* Please adjust according to custom specific protocol

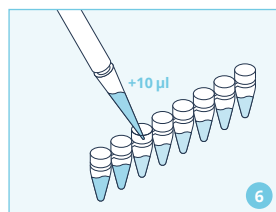
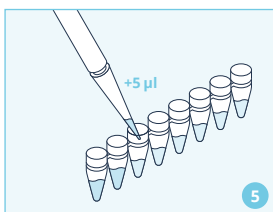
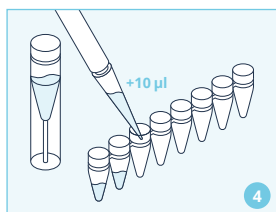
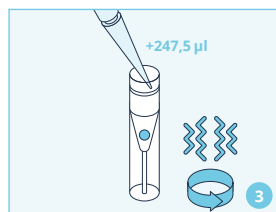
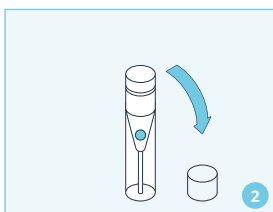
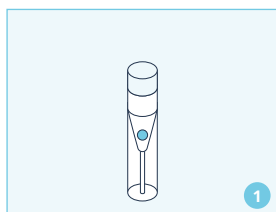
● Prepare DNA template as usual and provide in PCR-grade water or 10 mM Tris-HCl pH 8.0 in a final concentration of 2–100,000 cp/μl (e.g., 20–1,000,000 cp in 10 μl* sample volume)

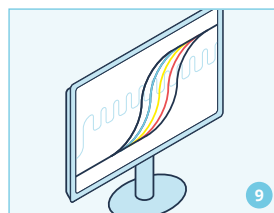
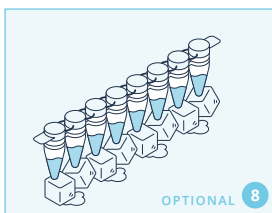
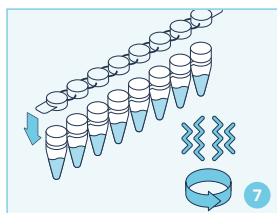
TAB. 2 Addition of template to bead-primer-probe-mix

Number of reactions	1
Bead solution (2.5x qPCR Master Mix)	10 μl
Primer-probe-Mix	5 μl*
Template*	10 μl*
Final volume	25 μl

* Please adjust according to custom specific protocol.

- 1 Take desired number of tubes with **Applyo qPCR Beads 25x Master Mix** 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 qPCR Beads 25x Master Mix** right before addition of Resuspension buffer for 25x MM Bead.
- 3 Reconstitute the Applyo Beads with 247,5 μl of Resuspension buffer for 25x MM Bead (Bead adds 2,5 μl to total volume = 250 μl final volume as a formulation of 2.5x qPCR Master Mix).
- 4 Add 10 μl resuspended Bead per well
- 5 Add 5 μl Primer-Probe-Mix per well
- 6 Add 10 μl Template to each tube





- 7 Seal tubes for PCR, Mix, 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*
95°C	1 min.
95°C	15 sec.
58°C	30 sec. repeat 45x*
Signal readout	

* Please adjust according to custom specific protocol.

4. Storage

Store 25x Beads at 20°C. Short term storage at temperatures ≤ 30°C is also possible.

Store 25x Bead-resuspension-buffer at 4°C. Resuspended Beads can be stored at -20°C for one week and used according to protocol above.

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

7. Application Specialist

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