

Check the product label for actual catalog number, lot and expiry date.

## Lyo-Ready 4X 1Step RT qPCR Probe Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP070LR-10	2000 r of 20 µl	2 x 5 ml – Lyo-Ready ORA™ qPCR Probe Mix, 4X 12.5 µl – Lyo-Ready RT7 Mix	Glycerol-free, Lyo-Ready qPCR Probe Mix, 4X contains Hot Start Taq, dNTPs, magnesium (4.5 mM final 1X conc.), buffer with excipients. The Lyo-Ready RT7 Mix (RNase Inhibitor+RTase) – is a highly concentrated blend of modified MMuLV RT and RNase Inhibitor formulated for lyophilization workflows with a minimized glycerol concentration.
QOP070LR-50	10 000 r of 20 µl	50 ml – Lyo-Ready ORA™ qPCR Probe Mix, 4X 62.5 µl – Lyo-Ready RT7 Mix	
QOP070LR-500	100 000 r of 20 µl	500 ml – Lyo-Ready ORA™ qPCR Probe Mix, 4X 625 µl – Lyo-Ready RT7 Mix	
<i>Storage</i>		<i>In the dark at -20°C.</i>	

### APPLICATIONS

- Development of lyophilized assays for virus/RNA detection
- Viral RNA detection in diluted low copy number samples
- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes

### PRODUCT DETAILS

4X 1Step RT qPCR Probe Kits are designed for a sensitive detection of specific RNAs, including virus RNA, in diluted high-volume samples. They combine a robust 4X qPCR mix with a blend of thermostable Reverse Transcriptase and RNase Inhibitor. This formulation allows for a high sample input volume with a reliable outcome of a single step RT qPCR when working with low copy number samples. The Lyo-ready version of the kit allows for the same sensitivity of RNA detection with a help of lyophilized reagents that can be conveniently stored and shipped without cooling. The Lyo-ready kit includes a 4X concentrated glycerol-free, Lyo-Ready ORA™ qPCR Probe Mix, 4X which contains Hot Start Taq, dNTPs, magnesium, in optimized buffer ready for lyophilization; and a highly concentrated Lyo-Ready RT7 Mix which is a blend of modified MMuLV RT and RNase Inhibitor optimized for lyophilization workflows due to minimized glycerol concentration in finally diluted solution.

### BENEFITS

- Glycerol-free, lyophilization-ready formulation of robust 4X qPCR mix for high sample volume input
- Highly concentrated blend of Reverse transcriptase and RNase Inhibitor for lyophilization workflows
- Detects <5 RNA copies, tested for Sars-CoV-2 detection
- Reverse transcription and qPCR in one tube
- Ideal for multiplex reactions and high throughput assays

### TECHNICAL DATA

The kit is suitable for preparing lyophilization beads or cakes in both plastic or glass vials, however, for each lyophilization workflow the conditions have to be optimized.

To distribute all components of the solution evenly, both components shall be mixed well after each thawing.

Lyo-Ready RT7 Mix is supplied in a small volume, therefore it is essential to spin it before the use to collect all drops.

Before lyophilization, the Lyo-Ready ORA™ qPCR Probe Mix, 4X shall be mixed with the Lyo-Ready RT7 Mix in a ratio 1 ml with 1.25 µl.

Both components have been tested for multiple freezing thawing cycles, and they are stable until the expiry date when stored at -20°C.

However, we do not recommend freezing the already mixed solution.

Mix both supplied components together right before the lyophilization. If required, the mix can be stored at +4°C for maximum 1-3 days.

### PROTOCOL

1. Thaw and keep reagents on ice. It is very important to mix them very well before use and to spin down all the drops!
2. Prepare the required volume of 1 Step RT qPCR solution by mixing each 1 ml of the Lyo-Ready ORA™ qPCR Probe Mix, 4X with the 1.25 µl of Lyo-Ready RT7 Mix (note, it is viscous). Provided component amounts are calculated to be mixed together by using total volume of each to prepare the lyo-ready 1 Step RT qPCR solution.
3. You can check the performance of the prepared liquid 1 Step RT qPCR solution by setting up the RT qPCR reaction as described in the example below. Alternatively, or afterwards go to step 4 to proceed with lyophilization. Keep the mixed solution cold at +4°C up to lyophilization.
  - ✓ Use 1-5 microliters of template or swab extract for 20 µl reaction.
  - ✓ 5 minutes are usually enough for reverse transcription at 45-55°C. For multiplex reactions, prolonged 10-20 min RT step is recommended.
  - ✓ Do not perform annealing/extension for more than 30 seconds. Use 58 °C temperature for this step. Optimization in a range 56 - 65°C is possible.
  - ✓ Prepare a 20 µl reaction:
 

Add your mixed 1 Step RT qPCR solution	5 µl
Add Reverse & Forward Primer	0.5 – 1 µM final conc. each
Add Specific Probe	130 – 500 nM final conc.
Add Template (RNA or crude sample)	1 – 5 µl (5 - 1x10 <sup>8</sup> copies)
Add PCR-grade Water	to 20 µl

 Mix gently, avoid bubbles. Place into the instrument set like:
 

Reverse Transcription	1 cycle: 55°C (45-55°C) for 5–10 (to 20) min.
RT inactivation/PCR activation	1 cycle: 95°C - 3 min
Denaturation	50 cycles: 95°C - 15 sec.
Annealing/extension	50 cycles: 58°C (55-65°C) - 30 sec.
  - ✓ Follow instrument instructions for melting curve analysis.

4. To proceed with lyophilization, after step 3, add Primers (0.5-1 µM final conc.) and Probe (130-500 nM final conc.).
5. For optimal lyophilization outcome, we recommend to dilute the final total reagent mix to a 1X - 2X concentration with nuclease free water.
6. Optionally, required ROX amount might be added prior to lyophilization. It shall not interfere with any of further processes.
7. Important temperature parameters:
  - Onset T<sub>c</sub> (collapsing) is -35.1°C / Onset T<sub>g</sub> (glass transition) is 68.9°C
  - Mid-point T<sub>g</sub> is 75.1°C / End-point T<sub>g</sub> is 81.3°C
8. The 0.5 ml of the mix has been tested for lyophilization in 2 ml glass vials. Optimization is required for other vials and instruments. For example, plastic PCR tubes will need less drying time.

#### Example of lyophilization conditions:

Step	Temp. °C	Time min.	Pressure	Explanation
Initialization 0	+4	NA	1.01325 bar	Load
Initialization 1	+5	10	1.01325 bar	Hold
Initialization 2	-50	110-220	1.01325 bar	Ramp 0.3-0.5°C/min
Initialization 3	-50	180	1.01325 bar	Hold
Primary drying 4	-45	10-20	0.03 mbar	Ramp 0.3-0.5°C/min
Primary drying 5	-45	5500	0.03 mbar	Hold
Secondary drying 6	+20 to +25	150	0.03 mbar	Ramp 0.3-0.5°C/min
Secondary drying 7	+20 to +25	600	0.03 mbar	Hold
Termination 8	+20 to +25	NA	0.507 bar	Backfill N <sub>2</sub>
Termination 9	+20 to +25	NA	0.507 bar	Stopper
Termination 10	+20 to +25	NA	1.01325 bar	Aerate

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