

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

## Lyo-Test 4X 1Step RT qPCR Probe Kit (to test prior lyophilization)

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP070LT-1	200 r of 20 µl	1 ml – Lyo-Ready ORA™ qPCR Probe Mix, 4X 0.2 ml – RT7 Mix, 20X	Glycerol-free, Lyo-Ready qPCR Probe Mix, 4X; it contains Hot Start Taq, dNTPs, magnesium (4.5 mM final 1X conc.), buffer with excipients. <i>NOTE: The Lyo-Ready kits have the same qPCR mix formulation.</i> RT7 Mix, 20X (RNase Inhibitor+RTase) is dedicated for testing (not for lyophilization), it is a 20X blend of modified MMuLV RT and RNase Inhibitor. <i>NOTE: The Lyo-Ready RT7 Mix included in lyo-ready kits has different formulation – it is higher concentrated (viscous) enzyme blend formulated for lyophilization with min. glycerol conc..</i>

Storage In the dark at -20°C.

### 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-testing kit is formulated to pre-evaluate the lyo-ready versions, it 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 RT7 Mix, 20X which is a blend of modified MMuLV RT and RNase Inhibitor used for pre-lyophilization testing.

### BENEFITS

- Glycerol-free, lyophilization-ready formulation of robust 4X qPCR mix for high sample volume input
- 20X concentrated blend of Reverse transcriptase and RNase Inhibitor for pre-lyophilization testing 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 testing the qPCR reaction performance prior to switching to the same Lyo-Ready Kit versions that are dedicated for lyophilization.

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

Before testing, the Lyo-Ready ORA™ qPCR Probe Mix, 4X shall be mixed with the RT7 Mix, 20X in a ratio 1 ml with 200 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 use. 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 0.2 ml of the RT7 Mix, 20X. Provided component amounts are calculated to be mixed together by using total volume of each to prepare the pre-lyo-test-ready 1 Step RT qPCR solution.
3. Optionally, required ROX amount might be added prior to reaction. It shall not interfere with any of further processes.
4. Keep the mixed solution cold at +4°C up to 1-3 days. Freezing is not recommended.
5. Check the performance of the prepared liquid 1 Step RT qPCR solution by setting up the RT qPCR reaction as described.
6. Use 1-5 microliters of RNA template or swab extract for 20 µl reaction.
7. 5 minutes are usually enough for reverse transcription at 45-55°C.
8. For multiplex reactions, a prolonged 10-20 min RT step is recommended.
9. Do not perform annealing/extension for more than 30 seconds. Use 58 °C temperature, optimize in a range of 56 - 65°C.

Set up 20 µl RT qPCR reaction:

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

IN VITRO RESEARCH USE ONLY

### ORDERING

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