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

4X 1Step RT qPCR Probe ROX L Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0501	200 r of 20 µl	1 ml - 1Step RT qPCR Probe ROX L Mix, 4X 0.2 ml – RT4 Mix, 20X (RNase Inhibitor+RTase) 1 ml - PCR Water	1Step RT qPCR Probe Mix, 4X - contains Hot Start Taq, dNTPs, optimized buffer, and low ROX concentration. RT4 Mix, 20X (RNase Inhibitor+RTase) - is a 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOP0505	1000 r of 20 µl	5 x 1 ml - 1Step RT qPCR Probe ROX L Mix, 4X 5 x 0.2 ml – RT4 Mix, 20X (RNase Inhibitor+RTase) 5 x 1 ml - PCR Water	
Storage	*In the dark at -20°C.*		

APPLICATIONS

- 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

BENEFITS

- Robust 4X qPCR mix for high sample volume input up to 10 µl
- Detects <5 RNA copies per reaction
- Successfully tested for Sars-CoV-2 detection
- Reverse transcription and qPCR in one tube
- Ideal for multiplex reactions
- Universal - both standard and fast cycling, GC/AT rich templates

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 20X 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 below 5 copies per reaction. 4X 1Step RT qPCR Probe Kits ensure robust performance of both reverse transcription and qPCR reactions, what allows for the highest sensitivity viral RNA detection under fast qPCR cycling conditions. The performance of the kits has been tested for Sars-CoV-2 detection in human specimens according to recommended Charité Berlin protocol with appropriate primers/probes.

PCR Water supplied in the kit ensures the best performance and reproducibility of the results.
Depending on your instrument requirements, the kit is available as no ROX, ROX L (low) and ROX H (high) versions.

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent it from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease free labware and gloves.
Whenever possible, before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- For controls, run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix them well before use!
- Use 2-5 or more microliters of swab extract for 20 µl reaction
- 5 minutes are enough for reverse transcription at 45-55°C. For multiplex reactions 10 min RT step might be required.
- Do not perform annealing/extension for more than 30 seconds. Use 58 °C temperature for this step. Optimization between 58 and 65°C is possible if needed.

✓ Prepare a 20 µl reaction:

Reverse Primer	0.5 – 1 µM final concentration
Forward Primer	0.5 – 1 µM final concentration
Specific Probe	150 – 500 nM final concentration
1Step RT qPCR Mix, 4X	5 µl
RT4 Mix, 20X	1 µl
Template (extracted RNA or crude sample from swabs)	2 – 5 µl (5 - 1x10^6 copies; you can add up to 7 - 10 µl in case of diluted RNA samples)
PCR Water	to 20 µl

✓ Mix gently, avoid bubbles.

✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 50°C (45-55°C) - 5 min
RT inactivation/PCR activation	1 cycle: 95°C - 3 min
Denaturation	50 cycles: 95°C - 15 sec.
Annealing/extension	50 cycles: 58°C (58-65°C) - 30 sec.

✓ Follow instrument instructions for melting curve analysis.

IN VITRO RESEARCH USE ONLY

ORDERING
T: +48 535 774 222
orders@eproscience.com

TECHNICAL SUPPORT
T: +48 535 774 222
eproscience@eproscience.com