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

2X 1Step RT qPCR Probe ROX H Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP1301	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Pro ROX H Mix, 2X 2 x 0.1 ml – RT-Pro Mix 2 x 1 ml - PCR Water	The 2X 1Step RT qPCR Pro RO L Mix includes Hot Start Taq DNA Polymerase, high conc. ROX and dNTPs in optimized qPCR buffer. RT-Pro Mix is an optimized blend of modified MMuLV RT and RNase Inhibitor in storage buffer.
QOP1305	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Pro ROX H Mix, 2X 10 x 0.1 ml – RT-Pro Mix 10 x 1 ml - PCR Water	
Storage	*In the dark at -20°C.*		

APPLICATIONS

- 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
- Detection of RNA viruses

BENEFITS

- Reverse transcription and qPCR in one tube
- Increased sensitivity, highest specificity
- Efficient cDNA synthesis from various template amounts
- Universal - both standard and fast cycling, GC/AT rich templates
- Excellent performance in multiplex assays

PRODUCT DETAILS

Our improved 2X 1Step RT qPCR Probe Kits provide highly sensitive target RNA detection when working with a wide variety of samples. The optimized 1Step RT qPCR Pro Mix in combination with a blend of thermostable Reverse Transcriptase and an advanced RNase Inhibitor (RT-Pro Mix) allows for a single step, one tube RT qPCR with great results even in multiplex reactions.

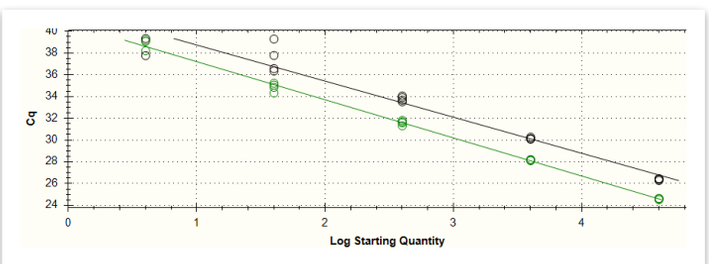
The novel RT-Pro Mix ensures safe and efficient RNA template conversion into a single-stranded cDNA. Pure RNA samples, as well as lysed crude samples can serve as templates for one-step RT qPCR. Though the kit was not specifically designed for crude sample qPCR, it has a potential to work well for this application.

The robust 1Step RT qPCR Pro Mix is a 2X concentrated qPCR master mix which includes dNTPs, buffer, and the hot start Taq DNA Polymerase. The Hot Start function ensures the highest sensitivity and specificity under both standard and fast qPCR cycling conditions.

The kits provide excellent results on both AT and GC-rich templates and show early Ct values with minimum or no optimization.

Our kits include PCR Water to guaranty the best performance. To suit the broad instrument range the 2X 1Step RT qPCR Probe Kits are available in three versions – without ROX, with low or high ROX concentration.

In most cases, the new kit (green) works better than the old kit (black). It provides better results with different template amounts (see below).



PROTOCOL

- Use special primer selection programs for experiment planning.
- Work with amplicons in a short range of 80-200, max 400 bp.
- Take typical measures to prevent RNA degradation and PCR contamination, keep your bench clean, wear gloves, use sterile tubes.
- If possible, check RNA template quality on agarose. Titrate the RNA template to choose the best concentration for quantification where the amplification curves with different template amount will be clearly separated from each other. If the template concentration is too low, the curves will stick together. For reproducible results, use higher template volumes of ~3-5 µl, or 5 µl swab extract for virus detection.
- Run reactions in triplets; include a no-template control, no RT Mix control and a trusted positive control in parallel.
- Thaw and keep reagents on ice. Mix them very well before use.
- Higher amounts of RT-Pro Mix may improve Ct, but primer dimers may appear. Titrate the best RT amount from 0.05 to 0.5 µl in 20 µl.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer (Tm ~60°C)	100-400 nM final c.
Forward Primer (Tm ~60°C)	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA template *or*	1 pg to 1 µg *or*
mRNA template *or*	>0.01 pg
viral RNA	10 to 10^7 copies
PCR Water	to 10 µl
1Step RT qPCR Pro ROX H Mix, 2X	10 µl
RT-Pro Mix	0.1-0.2 µl (from 0.05 to 0.5 µl)

✓ Mix gently, avoid bubbles.

✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 45°C (optimal) – 55°C (for complex templates) - 10 min
Initial denaturation	1 cycle: 95°C - 2 min
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60-65°C – 20-30 sec

✓ Follow instrument instructions for melting curve analysis.

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