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

1Step RT qPCR Probe ROX H Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0301	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Probe ROX H Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer, high ROX concentration. 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOP0305	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Probe ROX H Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer, high ROX concentration. 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
Storage	*In the dark at -20°C.*		

APPLICATIONS

- qPCR on instruments calibrated with high ROX conc.
- 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

- Reverse transcription & qPCR in one tube with highest sensitivity
- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
- Universal - both standard and fast cycling, GC or AT rich templates
- Rapid extension, early Ct

PRODUCT DETAILS

highQu 1Step RT qPCR mastermixes in combination with a blend of thermostable and extremely active Reverse Transcriptase & advanced RNase Inhibitor (RT Mix) allow for a single step one tube RT qPCR.
qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum optimization.
Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the 1Step RT qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.

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.

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.
- 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 well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
- 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	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA template *or* mRNA template	1 pg to 1 µg *or* >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1-2 µl

✓ Mix gently, avoid bubbles.

✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 40-55°C - 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.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

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