



qScript™ XLT One-Step RT-qPCR ToughMix®, ROX™

Cat. No. 95133-100 Size: 100 x 20- μ L reactions
95133-500 500 x 20- μ L reactions

Store at -20°C protected from light

Description

qScript XLT One-Step RT-qPCR ToughMix, ROX is a ready-to-use, highly sensitive master mix for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using hybridization probe detection chemistries such as TaqMan® 5'-hydrolysis probes on Applied Biosystems 7000, 7300, 7700, 7900HT StepOne™, or StepOnePlus™ instruments. First-strand cDNA synthesis and PCR amplification are carried out in the same tube without opening between procedures. It is ideal for highly sensitive quantification of RNA viruses or low abundance RNA targets as well as high throughput gene-expression studies. The system has been optimized to deliver maximum RT-PCR efficiency, sensitivity, and specificity in reduced reaction volumes and fast cycle times. qScript XLT One-Step RT-qPCR ToughMix, ROX contains all required components for RT-qPCR except RNA template and probe. It is compatible with all dual-labeled probe chemistries.

qScript XLT is an engineered M-MLV reverse transcriptase with reduced RNase H activity and improved activity and stability at higher temperatures. The use of higher temperatures (50 to 55°C) for the first-strand step of one-step RT-qPCR provides higher specificity for primer annealing and disruption of RNA secondary structure that can interfere with cDNA synthesis.

qScript XLT One-Step RT-qPCR ToughMix, ROX is highly resistant to PCR inhibitors. A key component of the ToughMix is an ultra pure, highly processive thermostable DNA polymerase that is combined with high avidity monoclonal antibodies. This provides an extremely stringent automatic hot-start that minimizes the potential for primer-dimer and other non-specific PCR artifacts. The light blue color of the AccuVue™ tracer dye simplifies reaction assembly in white, or clear, plates and helps to minimize pipetting or mixing errors. It does not interfere with qPCR performance or affect the stability of the product.

Instrument Compatibility

Different real-time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. It is critical to match the appropriate qPCR reagent to your specific instrument. The qScript XLT One-Step qRT-PCR ToughMix, ROX contains an internal reference dye that has been optimized for use on Applied Biosystems 7000, 7300, 7700, 7900HT StepOne™, or StepOnePlus™. Please visit our web site at www.quantabio.com to find an optimized kit for your instrument platform(s).

Components

| Reagent | Description |
|---|--|
| qScript XLT One-Step RT-qPCR ToughMix, ROX (2X) | 2X reaction buffer containing dATP, dCTP, dGTP, dTTP, magnesium chloride, qScript XLT reverse transcriptase, RNase inhibitor protein, hot-start DNA polymerase, AccuVue blue qPCR dye, ROX Reference Dye and stabilizers |

Storage and Stability

qScript XLT One-Step RT-qPCR ToughMix, ROX is stable for one year when stored in a constant temperature freezer at -20°C protected from light. Repeated freezing and thawing does not affect RT-qPCR performance.

Guidelines for One-Step RT-qPCR

- The design of highly specific primers and probes is a critical parameter for successful one-step RT-qPCR. The use of computer aided primer design programs is encouraged in order to minimize the potential for internal secondary structure and complementation at 3'-ends within each primer, the primer pair, and primer/probe combinations. Regions of strong RNA secondary structure should be avoided as this can interfere with primer hybridization and/or impede procession of the reverse transcriptase. For best results, amplicon size should be between 70 and 150 bp. Optimal results may require titration of primer concentration between 300 and 900 nM. A final concentration of 450 nM each primer and 100 to 150 nM probe is effective for most applications. The efficacy and efficiency of any primer/probe set should be validated under fast cycling and/or rapid ramp rate protocols before use in qPCR studies.
- If frozen, thaw qScript XLT One-Step RT-qPCR ToughMix, ROX on ice. Thoroughly mix by gently vortexing, and then centrifuge to collect contents to the bottom of the tube. Retain on ice before use.

Guidelines for One-Step RT-qPCR continued:

- To maximize specificity, reactions should be assembled on ice. The hot-start DNA polymerase is inactive prior to high temperature activation; however, qScript XLT reverse transcriptase is active at lower temperatures.
- First-strand synthesis can be carried out between 42°C and 55°C. Optimal results are generally obtained with a 5 to 10-minute incubation at 48 to 50°C. Longer incubation times for first-strand synthesis (up to 20 min) may be used.
- We recommend a minimum of 30s incubation at 95°C to inactivate the RT and activate the hot-start polymerase prior to PCR cycling. Longer activation times (2-10 minutes) will not adversely affect product performance.
- The kit is compatible with both fast or standard qPCR cycling protocols. Annealing and/or extension temperatures may need to be optimized for a given primer/probe design or fluorogenic probe chemistry. Use the suggested protocol as a starting point. Multiplexed RT-qPCR may benefit from a slightly longer extension time (45 to 60s).
- Preparation of a reaction cocktail is recommended to reduce pipetting errors and maximize assay precision. Assemble the reaction cocktail with all required components except RNA template and dispense equal aliquots into each reaction tube. Add RNA to each reaction as the final step. Addition of sample as 2 to 5-µL volumes will improve assay precision.
- Suggested input quantities of template are: 1 pg to 100 ng total RNA; 10 fg to 10 ng poly A(+) RNA; 10 to 1x10⁸ copies viral RNA.
- After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Reaction Assembly

| Component | Volume for 20-µL rxn. | Final Concentration |
|---|-----------------------|---------------------|
| qScript XLT One-Step RT-qPCR ToughMix, ROX (2X) | 10 µL | 1X |
| Forward primer | variable | 300 – 900 nM |
| Reverse primer | variable | 300 – 900 nM |
| Probe | variable | 50-200 nM |
| Nuclease-free water | variable | |
| RNA template | <u>2 to 5 µL</u> | variable |
| Final Volume (µL) | 20 µL | |

Note: For smaller, or larger, reaction volumes scale all components proportionally.

RT-qPCR Cycling Protocol

Incubate complete reaction mix in a real-time PCR detection system as follows:

| | |
|------------------------------|---|
| cDNA Synthesis | 50°C, 10 min |
| Initial denaturation | 95°C, 1 min |
| PCR cycling (30 - 45 cycles) | 95°C, 3s to 10s |
| | 60°C, 30s to 60s (data collection step) |

Quality Control

Kit components are free of contaminating DNase and RNase. qScript XLT One-Step RT-qPCR ToughMix, ROX is functionally tested in RT-qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range ($r^2 > 0.995$) and a PCR efficiency > 90%

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