



## UltraPlex™ 1-Step ToughMix® ROX™ (4X)

Cat No. 95167-100      Size: 100 x 20- $\mu$ L reactions (500  $\mu$ L)  
95167-500              500 x 20- $\mu$ L reactions (5 x 500  $\mu$ L)

**Store at -25°C to -15°C  
protected from light**

### Description

UltraPlex 1-Step ToughMix ROX is a ready-to-use, 4X-concentrated master mix for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using hybridization probe detection chemistries such as TaqMan® 5'-hydrolysis probes on real-time PCR systems that utilize ROX passive reference with 490 nM excitation. 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 in uni- or multiplexed RT-qPCR applications as well as high throughput gene-expression studies. The system has been optimized to deliver maximum RT-qPCR efficiency, sensitivity, and specificity in reduced reaction volumes and fast cycle times. UltraPlex 1-Step 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 (RT) with reduced RNase H activity for improved activity and stability at higher temperatures. The use of higher temperatures (50 to 54°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. These beneficial properties of qScript XLT RT are further enhanced by a hot-start mechanism for the reverse transcription step. Minimizing off-target extension by the RT during reaction assembly provides highly reproducible low copy quantification as well as extended room temperature stability of fully assembled reactions for high throughput operations.

UltraPlex 1-Step 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.

### 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. UltraPlex 1-Step ToughMix ROX provides seamless integration on the Applied Biosystems 7000, 7300, 7700, 7900HT StepOne™, or StepOnePlus™. Please visit our web site at [www.quantabio.com](http://www.quantabio.com) to find an optimized kit for your instrument platform(s).

### Components

Reagent	Description
UltraPlex 1-Step ToughMix ROX (4X)	4X reaction buffer containing dATP, dCTP, dGTP, dTTP, magnesium, qScript XLT reverse transcriptase, RNase inhibitor protein, hot-start DNA polymerase, stabilizers, and ROX Reference Dye

### Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C protected from light upon receipt. For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

### 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 400 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.

### Guidelines for One-Step RT-qPCR continued:

- If frozen, thaw UltraPlex 1-Step ToughMix ROX on ice. Thoroughly mix by vortexing, and then centrifuge to collect contents to the bottom of the tube. Retain on ice before use.
- First-strand synthesis can be carried out between 42°C and 60°C. Optimal results are generally obtained with a 10-minute incubation at 50°C. Longer incubation times for first-strand synthesis (up to 20 min) may be used. Incubation at temperatures over 54°C may result in delayed Cq's for assays that are optimal at 48 - 50°C.
- 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 and may reduce early cycle background noise experienced with some hydrolysis probe chemistries.
- 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 (60 to 90s). Use of a slower ramp rate (~2.5°C/s) between the denaturation step and annealing/extension step may improve performance for some assays.
- To maximize specificity, reactions should be assembled and retained on ice before transfer to the qPCR instrument.
- 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 mix thoroughly by vortexing**. Then, 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 1x10<sup>8</sup> copies viral RNA.
- After sealing each reaction, mix contents by vortexing, and then centrifuge briefly to collect components at the bottom of the reaction tube.

### Reaction Assembly

Component	Volume for 20-µL rxn.	Final Concentration
UltraPlex 1-Step ToughMix ROX (4X)	5 µL	1X
Forward primer(s)	variable	300 – 900 nM
Reverse primer(s)	variable	300 – 900 nM
Probe(s)	variable	50-200 nM
Nuclease-free water	variable	
RNA template	<u>2 to 10 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, 3 min
PCR cycling (30 - 45 cycles)	95°C, 3s to 10s
	60°C, 30s to 90s (data collection step) <sup>‡</sup>

<sup>‡</sup>Note: The use of longer extension times (90s at 60°C), or a 3-step cycling protocol with an extension step of 60s at 72°C can help mitigate suppression of a low copy amplicon when co-amplified with a high copy target sequence.

### Quality Control

Kit components are free of contaminating DNase and RNase. UltraPlex 1-Step ToughMix ROX is functionally tested in duplexed RT-qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range ( $r^2 \geq 0.990$ ) and a PCR efficiency  $\geq 90\%$  for the primary GOI with constant detection for the limiting exogenous positive control assay.

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