



qScript® One-Step qRT-PCR Kit, ROX™

Cat. No 95058-200 Size: 200 x 50-µL reactions

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

Description

The qScript One-Step qRT-PCR Kit, ROX is a convenient and highly sensitive solution for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using hybridization probe detection chemistries such as TaqMan® 5'-hydrolysis probes or molecular beacons on Applied Biosystems 7000, 7300, 7700, 7900HT, StepOne™, or StepOnePlus™ instruments. cDNA synthesis and PCR amplification are carried out in the same tube without opening between procedures. The system has been optimized to deliver maximum RT-PCR efficiency, sensitivity, and specificity, enabling unbiased co-amplification of low copy transcripts in the presence of higher copy reference genes. The proprietary reaction buffer has been specifically formulated to maximize activities of both reverse transcriptase and Taq DNA polymerase while minimizing the potential for primer-dimer and other non-specific PCR artifacts. Highly specific amplification is crucial to successful qRT-PCR as non-specific product(s) can compete for amplification of the target sequence and impair PCR efficiency. A key component of this kit is AccuStart Taq DNA polymerase, which contains monoclonal antibodies that bind to the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation at 95°C, the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase.

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 One-Step qRT-PCR Kit, ROX contains an internal reference dye that has been optimized for use on Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, StepOne, or StepOnePlus. Please consult the following table, or visit our web site at www.quantabio.com to find an optimized kit for your instrument platform(s).

Reagent	Cat Nos	Compatible Real-Time PCR Systems
qScript One-Step qRT-PCR Kit, ROX	95058-200	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
qScript One-Step qRT-PCR Kit, Low ROX	95059-200	Applied Biosystems 7500, 7500 Fast, ViiA™ 7 Stratagene MX4000™, MX3005P™, MX3000P™
qScript One-Step qRT-PCR Kit	95057-200	Bio-Rad CFX96™, CFX384™, iCycler iQ®, iQ™5, MyiQ™ Opticon™, MiniOpticon™, Chromo4™ Cepheid Smart Cycloer®; Qiagen/Corbett Rotor-Gene® Eppendorf Mastercycler® ep realplex Roche Applied Science LightCycler® 480

Components

Reagent	Description
qScript One-Step Reverse Transcriptase	Optimized 50X formulation of recombinant MMLV reverse transcriptase for one-step RT-PCR.
One-Step Master Mix, ROX (2X)	2X reaction buffer containing dNTPs, magnesium chloride, AccuStart Taq DNA polymerase, ROX Reference Dye, and stabilizers
Nuclease-free water	

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C protected from light upon receipt. Repeated freezing and thawing of the reaction mix is not recommended. For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.



Guidelines for One-Step qRT-PCR

- Thaw all components, except qScript One-Step RT, at room temperature. Mix vigorously, and then centrifuge to collect contents to the bottom of the tube before using. Place all components on ice after thawing.
- To maximize specificity, reactions should be assembled on ice. AccuStart Taq DNA polymerase is inactive prior to high temperature activation; however, qScript One-Step reverse transcriptase is active at lower temperatures. First-strand synthesis can be carried out between 42°C and 52°C. Optimal results are generally obtained with a 10-minute incubation at 48 – 50°C. We recommend a 5 minute incubation at 95°C to fully inactivate the RT prior to PCR cycling.
- 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 5 to 10-µL volumes will improve assay precision.
- Suggested input quantities of template are: 1 pg to 1 µg total RNA; 10 fg to 100 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 50-µL rxn.	Final Concentration
One-Step Master Mix, ROX (2X)	25 µl	1X
Forward primer	variable	400 – 900 nM
Reverse primer	variable	400 – 900 nM
Probe	variable	50-200 nM
Nuclease-free water	variable	
RNA template	5 – 10 µL	Variable
qScript One-Step RT	<u>1 µL</u>	1X
Final Volume (µL)	50 µL	

Note: For smaller reaction volumes (i.e. 25-µL reactions), scale all components proportionally.

Reaction Protocol

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

cDNA Synthesis	48 – 50°C, 10 min
Initial denaturation	95°C, 5 min
PCR cycling (30 - 45 cycles)	95°C, 10 to 15s
	55 – 60°C, 30 to 60s (data collection step)

Quality Control

Kit components are free of contaminating DNase and RNase. The qScript One-Step qRT-PCR Kit, 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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