qScript™ One-Step SYBR® Green qRT-PCR Kit, Low ROX™

Cat No. 95089-050 Size: 50 x 50-µL reactions
95089-200 200 x 50-µL reactions

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

Description

The qScript One-Step SYBR Green qRT-PCR Kit, Low ROX is a convenient and highly sensitive solution for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using SYBR Green I dye detection and gene-specific primers on Applied Biosystems 7500, 7500 Fast, ViiA™ 7, or Stratagene MX series of real-time PCR systems. 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-qPCR efficiency, sensitivity, and specificity. 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. The kit is compatible with both fast and standard qPCR cycling protocols. Highly specific amplification is essential for successful RT-qPCR with SYBR Green I technology, since this dye binds to any dsDNA generated during amplification. AccuStart™ Taq DNA polymerase contains monoclonal antibodies that bind to the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation at 95ºC, 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 and internal reference dye to your specific instrument. The qScript Custom One-Step SYBR Green qRT-PCR Kit, Low ROX provides seamless integration on the Applied Biosystems 7500, 7500 Fast, ViiA 7, or Stratagene MX series of real-time PCR systems. Please visit our web site at www.quantabio.com to find the optimal kit for your instrument platform.

Components

Reagent	Description	95088-050	95088-200
qScript One-Step Reverse Transcriptase	Optimized 50X formulation of recombinant MMLV reverse transcriptase for one-step RT-PCR.	1 x 50 µL	1 x 200 µL
One-Step SYBR Green Master Mix, Low ROX (2X)	2X reaction buffer containing dNTPs, magnesium chloride, AccuStart Taq DNA polymerase, stabilizers, ROX dye (for 580-585 nm excitation) and SYBR Green I dye	1 x 1.25 mL	4 x 1.25 mL
Nuclease-free water		1 x 1.5 mL	4 x 1.5 mL

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 SYBR Green qRT-PCR

- Primer design is critical for successful one-step RT-qPCR with SYBR Green. The use of software tools for PCR primer design and RNA secondary structure analysis can aide in the design of specific and efficient primers for one-step RT-qPCR. Primers should be designed according to standard qPCR guidelines with a length of 18 - 25 nucleotides and a GC content of 40-65%. Avoid internal secondary structure, and complementation at 3' ends within each primer and primer pair. 3'-end terminal stability should be kept low to maximize primer specificity (3'-pentamer ΔG^{o} > -8.0 kcal/mol or have no more than 2 to 3 Cs or Gs in the last 5 bases).
- Regions of RNA secondary structure should be avoided as this can interfere with annealing of the reverse primer for cDNA synthesis and/or impede procession of the reverse transcriptase. Programs for RNA structure prediction, such as the mfold web server (http://mfold.bioinfo.rpi.edu/), are useful for selecting regions of relaxed RNA structure for qRT-PCR primer design.
- Ideally, primer Tm should be between 58 and 60ºC for a typical 2-step qPCR cycling protocol. Estimation of primer Tm varies widely with different methods and analysis parameters. We recommend using a program that calculates Tm based on nearest-neighbor thermodynamic models at 50 mM monovalent salt and 50 nM primer concentration. Primers with melting temperatures outside of this range may require optimization of PCR cycling conditions.
- PCR product size should be between 70- 200 bp. Ideally, the amplified sequence should span intronic sequence to minimize the potential to amplify genomic DNA sequence. Design primers to anneal to exons that bracket intronic sequence or within exon / exon boundaries of the specific mRNA. NCBI's Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHomeAd) can facilitate the design of RNA-specific primer sets. Control reactions that lack reverse transcriptase (minus RT) should always be included to verify that amplification signal is due to the presence of RNA target and not genomic DNA.
- A final concentration of 200 nM each primer is recommended as a general starting point. Optimal results may require titration of primer concentration between 100 and 500 nM. PCR efficiency is often improved with higher primer concentration (300 to 500 nM). In some cases, higher concentration of the reverse primer alone may improve RT-PCR efficiency without compromising specificity. We highly recommend including a post PCR dissociation analysis step (melt curve) to distinguish specific from non-specific amplification product(s) (i.e. primer-dimer).
- Thaw all components, except the qScript One-Step RT, at room temperature. Mix by gently vortexing, then centrifuge to collect contents to the bottom of the tube before using. Place all components on ice after thawing.
- To maximize assay specificity and sensitivity reactions should be assembled on ice and kept cold until placed in your real-time PCR system. Centrifugation steps should be carried out in a refrigerated centrifuge. AccuStart Taq DNA polymerase is inactive prior to high temperature activation; however, reverse transcriptases are active at lower temperatures and can use single strand DNA as a template.

Guidelines for One-Step SYBR Green qRT-PCR continued:

- First-strand synthesis can be carried out between 42°C and 52°C. Optimal results are generally obtained with a 5-minute incubation at 50°C. We recommend a 2-5 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 100 ng total RNA; 10 fg to 100 ng poly A(+) RNA; 10 to $1x10^8$ 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 SYBR Green Master Mix, Low ROX (2X)	25 µL	1X
Forward primer	Variable	200 – 300 nM
Reverse primer	Variable	200 – 300 nM
Nuclease-free water	Variable	
RNA template	5 – 10 µL	Variable
qScript One-Step RT *	1 µL	1X
Final Volume (µL)	50 µL	

Note: Reaction volume can be scaled from 5 to 50 µL depending on the reaction plate (i.e. 384-well vs. 96-well) and qPCR system. Scale all component volumes proportionally. * Omit addition of qScript One-Step RT in minus RT control reactions.

Reaction Protocol

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

	Fast qPCR Cycling	Standard qPCR Cycling	3-Step PCR Cycling
cDNA Synthesis	50°C, 5 min	48 – 50°C, 10 min	48 – 50°C, 10 min
Taq Activation	95°C, 2 min	95°C, 5 min	95°C, 5 min
PCR cycling (30 - 45 cycles)	95°C, 3s 60°C, 30s (data collection)	95°C, 10s 60°C, 30s (data collection)	95°C, 10s 55 – 65°C, 20s 68 – 72°C, 30 to 60s (data collection)
Melt Curve (dissociation stage):	See instrument instructions	See instrument instructions	See instrument instructions

Optimal cycling conditions will vary for different primer sets. A 3-step cycling protocol may improve assay specificity with some primer sets.

Quality Control

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

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