qScript™ One-Step Fast qRT-PCR Kit

Cat. No. 95079-100 Size: 100 x 20-µL reactions
95079-500 500 x 20-µL reactions

Store at -20ºC protected from light

Description

The qScript One-Step Fast qRT-PCR Kit 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 real-time quantitative PCR systems that do not require an internal reference dye. 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.

The One-Step Fast Master Mix is provided as a 4X concentrated solution to allow addition of higher amounts of RNA template and improved detection sensitivity with low concentration samples. The unique formulation maximizes the activities of both reverse transcriptase and Taq DNA polymerase while minimizing the potential for primer-dimer and other non-specific PCR artifacts. This enables unbiased co-amplification of low copy transcripts in the presence of higher copy reference genes in duplexed qRT-PCR applications.

Highly specific amplification is crucial to successful RT-qPCR 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 during reaction assembly and the 50ºC reverse transcription step. A brief 30 second heat activation step at 95ºC irreversibly denatures the antibodies, releasing fully active, unmodified Taq DNA polymerase. Rapid recovery of fully active, unmodified Taq DNA polymerase is critical for efficient extension kinetics. Replication of fragments up to 200 bp is complete in less than 20s at 60ºC. The qScript One-Step Fast qRT-PCR Kit affords greater reagent economy and laboratory throughput on conventional or rapid ramp rate qPCR systems.

For minor groove binder (MGB) modified probes, we recommend the qScript One-Step Fast MGB qRT-PCR Kit.

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 Fast qRT-PCR Kit does not contain an internal reference dye. 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 Fast qRT-PCR Kit, ROX	95080-100, 95080-500	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
qScript One-Step Fast qRT-PCR Kit, Low ROX	95081-100, 95081-500	Applied Biosystems 7500, 7500 Fast, ViiA™ 7 Stratagene MX4000™, MX3005P™, MX3000P™
qScript One-Step Fast qRT-PCR Kit	95079-100, 95079-500	Bio-Rad CFX96™, CFX384™,iCycler iQ®, iQ™5, MyiQ™ Opticon™, MiniOpticon™, Chromo4™ Cepheid Smart Cycler®; Qiagen/Corbett Rotor-Gene® Eppendorf Mastercycler® ep realplex Roche Applied Science LightCycler® 480

Components

Reagent	Description
qScript One-Step Fast RT	Optimized 20X formulation of recombinant MMLV reverse transcriptase for one-step Fast qRT-PCR.
One-Step Fast Master Mix (4X)	4X reaction buffer containing dNTPs, magnesium chloride, AccuStart Taq DNA polymerase, and stabilizers
Nuclease-free water	

Storage and Stability

Kit components are stable for one year when stored in a constant temperature freezer at -20ºC protected from light. For convenience, the One-Step Fast Master Mix may be stored unfrozen at +2 to +8ºC for up to 6 months. Repeated freezing and thawing of the reaction mix is not recommended.

Guidelines for One-Step qRT-PCR

- 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 RT-qPCR studies.
- Thaw all components, except qScript One-Step Fast RT, at room temperature. Mix vigorously, 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 5-minute incubation at 48 – 50°C. We recommend a minimum of 30s incubation at 95°C to inactivate the RT and activate AccuStart Taq 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 $1x10^8$ 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 20-µL rxn.	Final Concentration
One-Step Fast Master Mix (4X)	5 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 to 10 µL	variable
qScript One-Step Fast RT	1 µL	1X
Final Volume (µL)	20 µL	

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

Reaction Protocol

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

cDNA Synthesis	48 – 50°C, 5 min
Initial denaturation	95°C, 30s
PCR cycling (30 - 45 cycles)	95°C, 3s
	60°C, 20 to 30s (data collection step)

Quality Control

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

Limited Label Licenses

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,804,375, 5,538,848, 5,723,591, 5,876,930, 6,030,787 and 6,258,569. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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