qScript™ One-Step qRT-PCR Kit

Cat. No: 95057-050 Size: 50 x 50-µL reactions **Store at -20°C**
95057-200 200 x 50-µL reactions

Description

The qScript One-Step 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. 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 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 to your specific instrument. The qScript One-Step 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 qRT-PCR Kit, ROX	95058-050 95058-200	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
qScript One-Step qRT-PCR Kit, Low ROX	95059-050 95059-200	Applied Biosystems 7500, 7500 Fast, ViiA™ 7 Stratagene MX4000™, MX3005P™, MX3000P™
qScript One-Step qRT-PCR Kit	95057-050 95057-200	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 Reverse Transcriptase	Optimized 50X formulation of recombinant MMLV reverse transcriptase for one-step RT-PCR.
One-Step Master Mix (2X)	2X 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. For convenience, the One-Step 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

- 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 $1x10^8$ 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 Master Mix (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	1 µL	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 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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