qScript™ One-Step Fast MGB qRT-PCR Kit

Cat. No. 95082-100 Size: 100 x 20-µL reactions **Store at -20°C protected from light**
95082-500 500 x 20-µL reactions

Description

The qScript One-Step Fast MGB qRT-PCR Kit is a convenient and highly sensitive solution for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using TaqMan®-MGB 5'-hydrolysis probes 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-qPCR efficiency, sensitivity, and specificity in reduced reaction volumes and fast cycle times.

The use of minor groove binder (MGB) modified probes can present unique challenges for effective one-step RT-qPCR. The qScript One-Step Fast MGB qRT-PCR Kit has been specifically optimized to provide exceptional RT-qPCR efficiency and sensitivity with TaqMan-MGB probes, such as TaqMan Gene Expression Assays from Applied Biosystems. The novel composition of the One-Step Fast MGB Master Mix maximizes the activities of both the reverse transcriptase and Taq DNA polymerase to allow unbiased amplification of low copy transcripts in the presence of high copy reference genes. The kit is suitable for use in duplexed one-step RT-qPCR with VIC™-MGB labeled TaqMan Endogenous Controls from Applied Biosystems. For conventional TaqMan probes that use either TAMRA™, Black Hole Quencher® (including BHQplus™), or molecular beacons, we recommend the qScript One-Step Fast qRT-PCR Kit.

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 50°C reverse transcription step. A brief 30-second heat activation step at 95°C 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 60°C. The qScript One-Step Fast MGB qRT-PCR Kit affords greater reagent economy and laboratory throughput on conventional or rapid ramp rate qPCR systems.

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 MGB 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 MGB qRT-PCR Kit, ROX	95083-100, 95083-500	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
qScript One-Step Fast MGB qRT-PCR Kit, Low ROX	95084-100, 95084-500	Applied Biosystems 7500, 7500 Fast, ViiA™ 7 Stratagene MX4000™, MX3005P™, MX3000P™
qScript One-Step Fast MGB qRT-PCR Kit	95082-100, 95082-500	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 MGB RT	Optimized 20X formulation of recombinant MMLV reverse transcriptase for one-step Fast qRT-PCR using MGB-modified probes.
One-Step Fast MGB 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 -20°C protected from light. For convenience, the One-Step Fast MGB Master Mix may be stored unfrozen at +2 to +8°C 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 qRT-PCR. 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. TaqMan Gene Expression Assays (FAM-MGB probe) should be used at 0.5X to 1X final concentration. For duplexed RT-qPCR with VIC-MGB labeled Endogenous Controls, we recommend using 0.5X final concentration of the primer/probe mix. The efficacy and efficiency of any primer/probe set should be validated before use in RT-qPCR studies.
- Thaw all components, except qScript One-Step Fast MGB 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 Fast MGB 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 MGB 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 MGB 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 MGB 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

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