qScript® Virus 1-Step ToughMix®

Cat No.	95131-100	Size:	100 x 20 µL reactions (1 x 1 mL)	**Store at -25°C to -15°C**
	95131-500		500 x 20 µL reactions (5 x 1 mL)	
	95131-02K		2000 x 20 µL reactions (1 x 20 mL)	

Description

qScript 1-Step Virus ToughMix is a 2x, ready-to-use, master mix for rapid detection of RNA viruses such as Flu-A, Flu-B, and SARS-CoV-2, using one-step, or single-tube reverse transcription quantitative PCR (RT-qPCR). It has been optimized for maximum sensitivity to enable reliable quantification of very low input quantities of RNA using dual-labeled hydrolysis probe detection chemistries such as TaqMan® probes in single or multiplexed assay formats. qScript 1-Step Virus ToughMix contains all required components for RT-qPCR except RNA template and primer/probe assays.

qScript 1-Step Virus ToughMix is powered by an engineered reverse transcriptase with reduced RNase H activity and improved activity and stability at higher temperatures, that includes a RT Hot-Start technology to suppresses non-specific primer extension by the RT before cDNA synthesis. The use of higher temperatures (50°C to 55°C) for the first-strand step of one-step RT-qPCR provides higher specificity for primer annealing and disruption of RNA secondary structure that can interfere with cDNA synthesis. These features combined with a stringent mAb hot-start Taq polymerase and ToughMix reagent technology to neutralize many common PCR inhibitors, provide reproducible low-copy quantification as well as extended room temperature stability of fully assembled reactions for automated reaction assembly. The light blue color of the AccuVue tracer dye simplifies reaction assembly in white, or clear, plates and helps to minimize pipetting or mixing errors. It does not interfere with qPCR performance or affect the stability of the product.

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. qScript 1-Step Virus ToughMix does not contain a passive reference dye.
Please visit our web site at www.quantabio.com to find an optimized kit for your instrument platform(s).

Components

Reagent	Description
qScript 1-Step Virus ToughMix (2X)	2X reaction buffer containing dATP, dCTP, dGTP, dTTP, magnesium chloride, qScript XLT reverse transcriptase, RNase inhibitor protein, hot-start DNA polymerase, AccuVue blue qPCR dye, and stabilizers

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C.
Repeated freezing and thawing do not affect RT-qPCR performance.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Guidelines for One-Step RT-qPCR

- 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 bp and 150 bp. Optimal results may require titration of primer concentration between 300 nM and 900 nM. Final concentrations of 450 nM each primer and 100 nM to 150 nM probe are 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 qPCR studies.

Guidelines for One-Step RT-qPCR continued:

- Thaw each component and mix by gently vortexing. Centrifuge to collect contents to the bottom of the tube. Retain on ice before use.
- The RT-hot-start mechanism suppresses non-specific primer extension by the reverse transcriptase at ambient temperatures; however, to maximize specificity, reactions should be assembled on ice. The qPCR system should be pre-programed and in a ready state to initiate the run.
- First-strand synthesis can be carried out between 42°C and 55°C. Optimal results are generally obtained with a 5 to 10-min incubation at 48°C to 50°C.Longer incubation times for first-strand synthesis (up to 20 min) may be used.
- Reverse transcriptase heat-kill and activation of the hot-start polymerase prior to PCR cycling is complete within 30s at 95°C. Longer activation times (2-3 min) generally provide more stable fluorescent baselines and eliminate aberrant automated baseline interval determinations.
- The kit is compatible with either fast, or standard qPCR cycling protocols. Annealing and or extension temperatures may need to be optimized for a given primer/probe design or fluorogenic probe chemistry. Use the suggested protocol as a starting point. Multiplexed RT-qPCR may benefit from a slightly longer extension time (45 to 60s).
- 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 2 to 5 µL volumes improves assay precision.
- Suggested input quantities of template are: 1 pg to 100 ng total RNA; 10 fg to 10 ng poly A(+) RNA; 10 to 1x108 copies viral RNA.
- After sealing each reaction, vortex gently to thoroughly mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Reaction Assembly

Component	Volume for 20-µL rxn.	Final Concentration
qScript Virus 1-Step ToughMix (2X)	10 µL	1X
Forward primer	variable	300 – 900 nM
Reverse primer	variable	300 – 900 nM
Probe	variable	50-200 nM
Nuclease-free water	variable	
RNA template	2 to 5 µL	variable
Final Volume (µL)	20 µL	

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

RT-qPCR Cycling Protocol

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

cDNA Synthesis	50°C, 10 min
Initial denaturation	95°C, 1 min
PCR cycling (30 - 45 cycles)	95°C, 3s to 10s
	60°C, 30s to 60s (data collection step)

Quality Control

Kit components are free of contaminating DNase and RNase. qScript 1-Step Virus ToughMix 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 signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this manual and for use with components contained in the kit only. QIAGEN Beverly, LLC. grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this manual, and additional protocols available at www.quantabio.com. Some of these additional protocols have been provided by Quantabio product users. These protocols have not been thoroughly tested or optimized by QIAGEN Beverly, LLC. QIAGEN Beverly, LLC. neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN Beverly, LLC. makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN Beverly, LLC. specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN Beverly, LLC. may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

Quantabio brand products are manufactured by QIAGEN, Beverly LLC.
Intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

Trademarks

qScript and ToughMix are registered trademark of QIAGEN Beverly, LLC. TaqMan is a registered trademark of Roche Molecular Systems, Inc.