

eQo 1-Step ToughMix®

Cat No.	95301-100	Size	100 x 20 µl reactions (500 µl)
	95301-01K		1000 x 20 µl reactions (5 ml)

Store dried at 2°C to 25°C
After rehydration store at -25°C to -15°C

Description

eQo 1-step ToughMix is a lyophilized reagent system for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using hybridization probe detection chemistries such as TaqMan® 5'-hydrolysis probes on systems that do not require a passive reference dye. It is supplied with a proprietary rehydration buffer that produces a stabilized 4X concentrated master mix that contains a thermolabile UDG for amplicon carryover elimination, an enhanced warm start reverse transcriptase (RT) and all other required components for 1-step RT-qPCR except RNA template and primer/probe(s). The reaction chemistry has been optimized for inhibitor tolerance and delivers exceptional performance in either singleplex or highly demanding multiplex 1-step RT-qPCR formats.

The included qScript® Ultra reverse transcriptase is an RNase H deficient retrovirus RT engineered for rapid and processive first-strand synthesis at temperatures up to 65°C (optimal 55°C to 60°C), which disrupts interfering RNA secondary structure and improves primer specificity. This novel RT is further enhanced by an aptamer warm start component that effectively blocks RT activity during reaction setup enabling highly sensitive and reproducible low copy quantification and extended room-temperature stability of fully assembled reactions. **To fully overcome the warm start suppression of activity, the reverse transcription step must take place at 55°C - 60°C.**

This robust 1-Step ToughMix has been processed and dried into small, stable beads, packaged into tubes, and sealed in pouches with desiccant packets to provide maximal stability for room-temperature shipping and storage. Upon receipt the sealed packets should be stored refrigerated at 2 - 8°C, or at ambient temperature ($\leq 25^\circ\text{C}$). **Once a sealed packet is opened, the entire quantity of dried beads must be immediately rehydrated using the provided buffer.** This rehydration buffer contains components that improve detection of GC-rich targets and additional stabilizers that allow for standard storage at -20°C of the 4X concentrated liquid ToughMix.

Components

Component Description	95301-100	95301-01K
Master Mix Pouch (contains 1 tube of lyophilized beads plus a desiccant packet)	100 reaction packet	1000 reaction packet
Rehydration Buffer	1 x 0.55 ml	1 x 5.5 ml

Storage and handling

Prior to rehydration:

Store all components refrigerated at 2°C to 8°C or at ambient temperature ($\leq 25^\circ\text{C}$) until kit expiration date. **Do not open the internal silver Master Mix pouch until ready to rehydrate the lyophilized beads using the included buffer.** See below for the rehydration protocol.



For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

After rehydration:



Store the 4X-concentrated 1-step ToughMix at -20°C (-25°C to -15°C). The rehydrated ToughMix is stable at this temperature and withstands up to 20 freeze-thaw cycles without compromised functional performance. After thawing, mix gently, but thoroughly by vortexing. Centrifuge briefly to collect contents before use.

Rehydration

eQo 1-Step ToughMix should be fully rehydrated in a single step. **Do not remove individual beads for smaller-scale rehydration.** Allow the foil packet labeled Master Mix Pouch to equilibrate to room temperature if necessary, then tear open the foil packet and remove the screw-capped tube containing the master mix beads. Gently tap the tube on a hard surface to ensure that no beads are stuck to the cap, then unscrew and accurately add 500 µl (100 reaction packet) or 5.0 ml (1000 reaction packet) of the provided rehydration buffer using an appropriate pipette. Screw the cap back onto the tube and gently tap if necessary to ensure that all beads are exposed to the rehydration buffer. Wait at least 3 minutes to allow the beads to fully rehydrate, then vortex for a few seconds and briefly centrifuge to consolidate the contents.

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 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 300 and 900 nM. A final concentration of 450 nM of each primer and 100 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.
- If frozen, thaw the 1-Step ToughMix at ambient temperature (20 to 22°C). Thoroughly mix by vortexing, and then centrifuge to collect contents to the bottom of the tube. Retain on ice before use.
- **First-strand synthesis should be carried out between 55°C and 60°C.** Optimal results are generally obtained with a 10-minute incubation. Longer incubation times for first-strand synthesis (up to 20 min) may be used.
- A 1 to 2 min incubation at 95°C is required to inactivate the RT and activate the hot-start polymerase prior to PCR cycling.
- The kit is compatible with both fast and 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. Use of a slower ramp rate (~2.5°C/s) between the denaturation step and annealing/extension step may improve performance for some assays.
- While it is acceptable to setup reactions on ice, assembly at room temperature is permissible. Due to the stringent RT warm start, rigid requirements for reaction assembly and retention on ice before transfer to the qPCR instrument are unnecessary.
- 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 mix thoroughly by vortexing. Then, 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 will improve 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, mix contents by vortexing, then centrifuge briefly to collect components at the bottom of the reaction tube.



Standard Reaction Assembly

Component	Volume for 20 µl rxn	Final Concentration
Nuclease-free water	variable	variable
Template RNA	2 to 10 µl	variable
4X eQo 1-Step ToughMix	5 µl	1X
Forward primer(s)	variable	300-900 nM
Reverse primer(s)	variable	300-900 nM
Probe(s)	variable	50-200 nM
Final volume	20 µl	

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

Cycling Protocol

Carryover elimination (optional)	25°C, 5 min
cDNA synthesis	55°C to 60°C , 10 min
Initial denaturation	95°C, 1 - 2 min
PCR cycling (30 - 45 cycles)	95°C, 3 – 10 s
	55°C to 62°C, 20 – 45 s

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. Quantabio, 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 Quantabio, LLC. Quantabio, LLC. neither guarantees them nor warrants that they do not infringe the rights of third parties.
2. Other than expressly stated licenses, Quantabio, 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. Quantabio, 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. Quantabio, 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.
6. The use of this product is covered by at least one claim of U.S. Patent No. 7,687,247 owned by Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product, (b) its components, or (c) materials made by the employment of this product or its components to a third party or otherwise use this product or its components or materials made by the employment of this product or its components for Commercial Purposes. Commercial Purposes means any activity for which a party receives or is due to receive consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. The buyer cannot use this product or its components or materials made using this product or its components for therapeutic, diagnostic or prophylactic purposes. Further information on purchasing licenses under the above patents may be obtained by contacting the Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008. Email: outlicensing@lifetech.com.



©2024 Quantabio, LLC. 100 Cummings Center Suite 407J Beverly, MA 01915 Telephone number: 1-888-959-5165. Quantabio products are manufactured in Beverly, Massachusetts, Frederick, Maryland and Hilden, Germany. 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 Quantabio, LLC.
TaqMan® is a registered Trademark of Roche Molecular Systems.