eQo 1-Step ToughMix®

Cat No.	95301-100	Size	100 x 20 µl reactions (500 µl)	**Store dried at 2°C to 25°C**
	95301-01K		1000 x 20 µl reactions (5 ml)	**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 (≤ 25°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 dessicant 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 (≤ 25°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 $1x10^8$ 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

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