eQo 1-Step ToughMix®

No compromises.
Unparalleled performance meets sustainability

FEATURES AND BENEFITS:

- Unrivaled Sensitivity – Detection down to 2 copies of RNA
- Sustainable & Earth Friendly – lyophilized eQo bead format eliminates dry ice, CO_2 consumptions & reduces shipping costs
- Novel Reverse Transcriptase – convenient, ambient reaction setup. Overcomes rigid RNA secondary structure
- High Stability – Up to 1 year at room temperature, up to 1 year rehydrated at –20°C
- Tough Tested – Broad and significantly enhanced tolerance to PCR inhibitors

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. It is supplied with a proprietary rehydration buffer that when combined with the lyophilized "eQo beads" produces a stabilized 4X concentrated master mix. The kit includes 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 single-plex or highly demanding multiplex 1-step RT-qPCR formats.

Enhanced Thermostability

The included qScript Ultra reverse transcriptase supports 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.

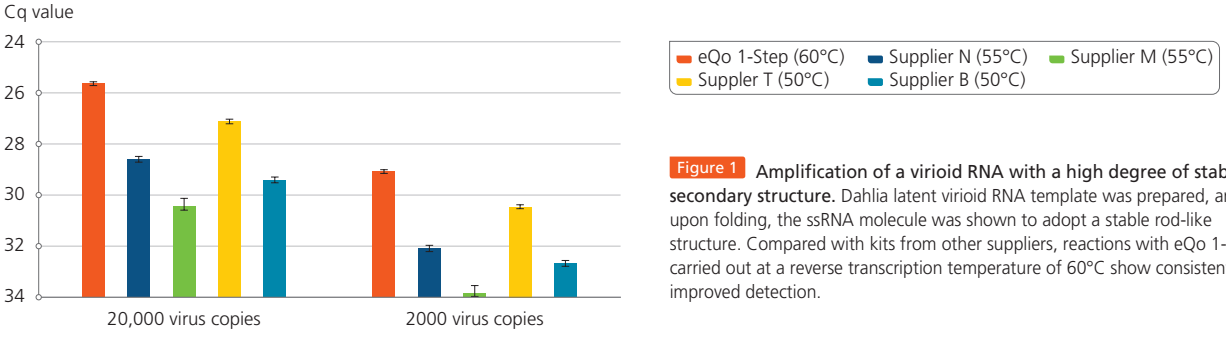
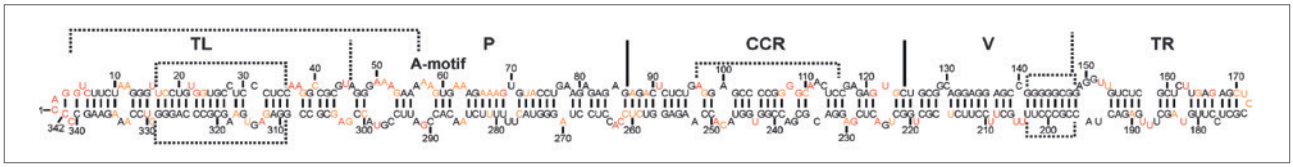


Figure 1 **Amplification of a virioid RNA with a high degree of stable secondary structure.** Dahlia latent virioid RNA template was prepared, and upon folding, the ssRNA molecule was shown to adopt a stable rod-like structure. Compared with kits from other suppliers, reactions with eQo 1-step carried out at a reverse transcription temperature of 60°C show consistently improved detection.



Overcome Challenging Samples

eQo 1-Step ToughMix demonstrates a tolerance to a broad range of common PCR inhibitors, reducing the requirement for perfectly "clean" samples. Additionally, amplification of difficult gene targets, include those that are GC-rich, is robust and consistent.

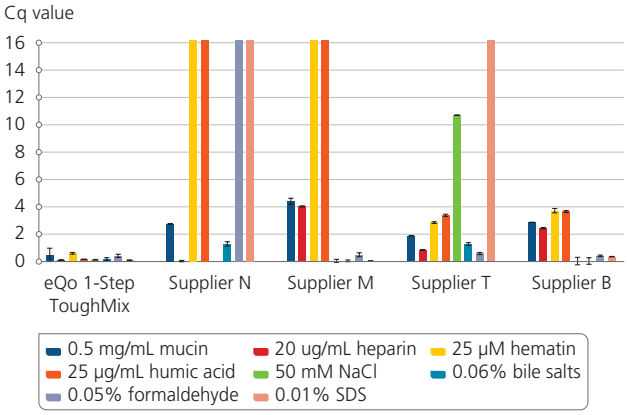


Figure 2 **Inhibitor resistance across wide range of sample types.** Innate inhibitor tolerance of qScript Ultra reverse transcriptase together with the formulation of the eQo 1-step mix allow for superior tolerance to many common reaction inhibitors. Reverse transcription was carried out at manufacturer's recommendations (including 60°C for eQo 1-Step); thermocycling was performed at the same cycling protocols for all. Cq values were compared with reactions without inhibitors to show relative inhibitor tolerance in the 1-step reaction.

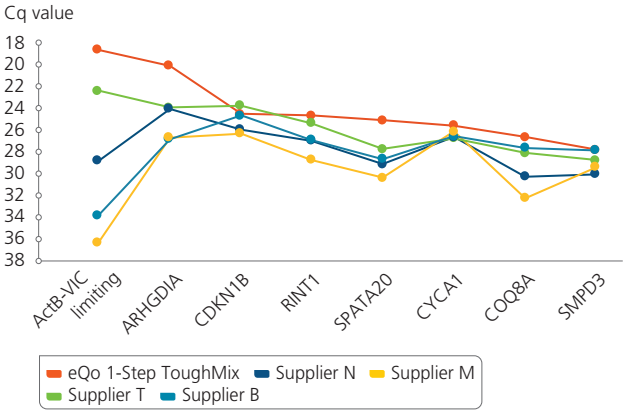


Figure 3 **Gene expression quantification using primer limiting of GC-rich targets. eQo 1-step shows consistent mRNA quantification over a range of difficult gene targets.** 1-step reaction mixtures were assembled in duplicate using 50 ng total human RNA and the indicated primer/probe assay sets. Reverse transcription was carried out at manufacturer's recommendations (including 60°C for eQo 1-Step); thermocycling was performed at the same cycling protocols for all The average Cq was plotted for each gene expression assay.

Extreme Sensitivity

Detection of extremely low-copy RNA virus is possible with eQo 1-Step ToughMix. Amplification of just 2 copies of SARS-CoV-2 RNA template is robust and clearly evident.

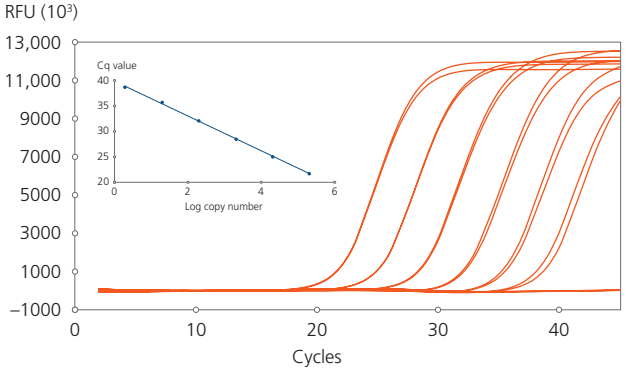


Figure 4 **Improved limits of detection for RNA pathogens: In a fourplex RT-qPCR experiment, eQo 1-step ToughMix can detect RNA pathogens in a purified sample matrix with as few as two copies.** A representative amplification plot was generated by diluting SARS-CoV-2 RNA from 200,000 to 2 copies. The inset plot displays the average Cq against the log copy number of the SARS-CoV-2 target. Strong amplification was observed even at the highest dilution.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
eQo 1-Step ToughMix - 100 R	95301-100	100 x 20 µL rxns
eQo 1-Step ToughMix - 1000 R	95301-01K	1000 x 20 µL rxns

MK-SF-0048 REV 03 eQo 1-Step ToughMix 0525