Two-Step RT-qPCR

UltraMarathonRT

RNAConnect

Unparalleled Performance, Improving and Simplifying Gene Expression Analysis

The **UltraMarathonRT® Two-Step RT-qPCR Kit** sets a new standard in RNA quantification, delivering unmatched sensitivity, reliability, and robustness for researchers. Most RT enzymes used in RT-qPCR have low processivity and are only able to cover relatively unstructured regions, which restricts qPCR primer design to limited regions of the transcript. Importantly, even when sophisticated software is used for primer design, different primer pairs can result in dramatically different C_t values for the same target when using non-processive RTs. This variability introduces uncertainty as to whether true gene expression levels have been identified by RT-qPCR or not. The new UltraMarathonRT RT-qPCR Kit solves this problem.

UltraMarathonRT provides Reliable Quantification Regardless of qPCR Primer Location

With intrinsic helicase activity, UltraMarathonRT (uMRT) swiftly navigates high GC content and RNA secondary structures, providing full-length cDNA synthesis. Having full-length cDNA as a template for the qPCR step enables easy primer pair selection along any section of the transcript without having to worry about sequence or structure-associated hurdles.

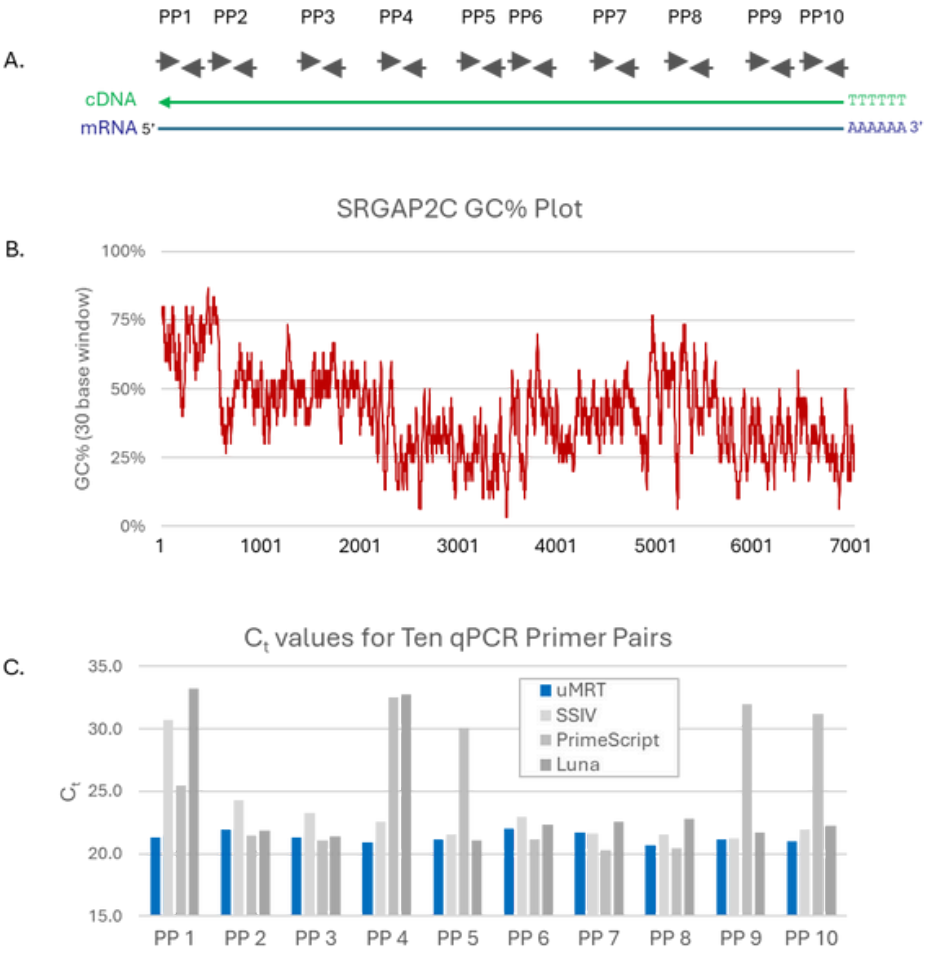

Figure 1. A) Diagram of the relative locations of the 10 primer pairs used for qPCR. B) Distribution of GC% for a 30-base window across the SRGAP2C gene. The GC% ranges from very high (>75%) to very low (<10%). C) The C_t values for four different RT-qPCR kits across all 10 primer pairs. Because the uMRT kit shows no 5' to 3' bias and no bias for GC content, the results of the qPCR are very consistent. The competing kits have highly variable results.

Gene-Specific RT Primers and Random Primers Finally Give the Same Results

The **UltraMarathonRT Two-Step RT-qPCR Kit** can be used with oligo(dT), gene-specific, or random primers. UltraMarathonRT ensures unbiased cDNA synthesis for accurate quantification. When doing RT-qPCR with MMLV RTs, random primers (randomers) may improve the detection of difficult templates, but their performance is still strongly affected by RNA sequence and structure. In addition, randomers generate multiple cDNA copies from a single RNA template, which strongly biases gene quantification by RT-qPCR. However, with uMRT, the reverse transcription product is dominated by the longest cDNA product generated using random primers, which allows accurate gene quantification.

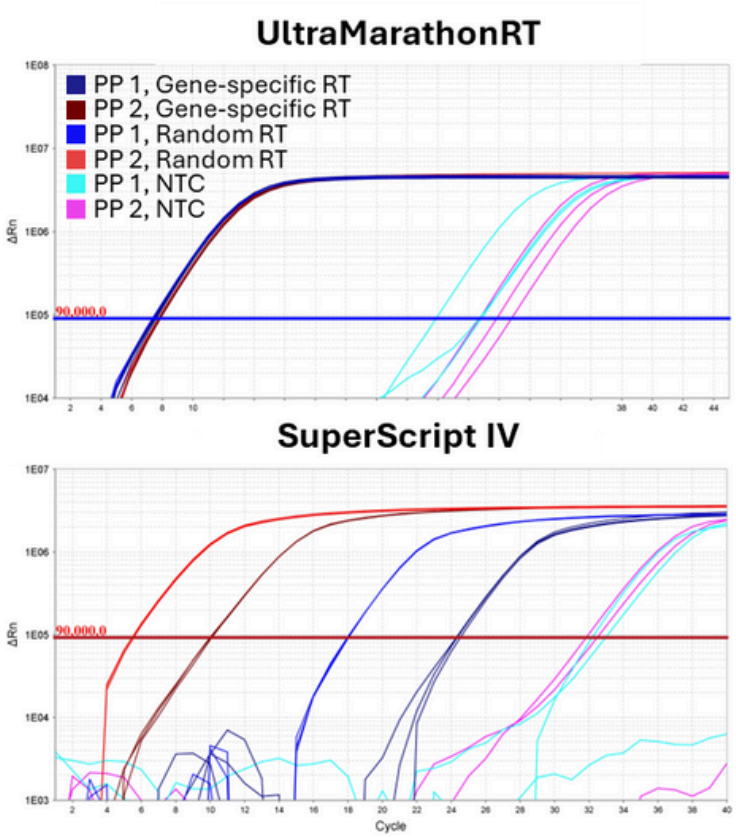

Figure 2. In this study, we compared random primers to gene-specific primers using UltraMarathonRT (uMRT) and SuperScript IV (SSIV). The reported qPCR primer pairs used were designed and optimized to identify eukaryotic-specific abundance of 28s rRNA (Kounosu et al. 2019). We chose two primer pairs that were close in amplicon location, size, and GC content. The **UltraMarathonRT Two-Step RT-qPCR Kit** consistently and reliably quantified the 28s rRNA using either gene-specific primers or random primers, while the SSIV Kit provided highly variable results depending on the type of RT primer used and qPCR primer pair location. NTC denotes no template control.

UltraMarathonRT Has a Broad Dynamic Range Delivering Accurate Quantification of All RNAs

When identifying and quantifying gene expression differences, it is essential to be able to compare transcripts, whether highly expressed or rare. This kit excels in detecting a wide range of RNA types, including low abundance, viral, non-coding, long, and structured RNAs. Its sensitivity and efficiency make it indispensable for complex RNA studies, accommodating RNA input concentrations from an 8-log range (0.1 pg to 2 µg). With such a broad dynamic range and high efficiency, the kit supports diverse experimental needs with ease.

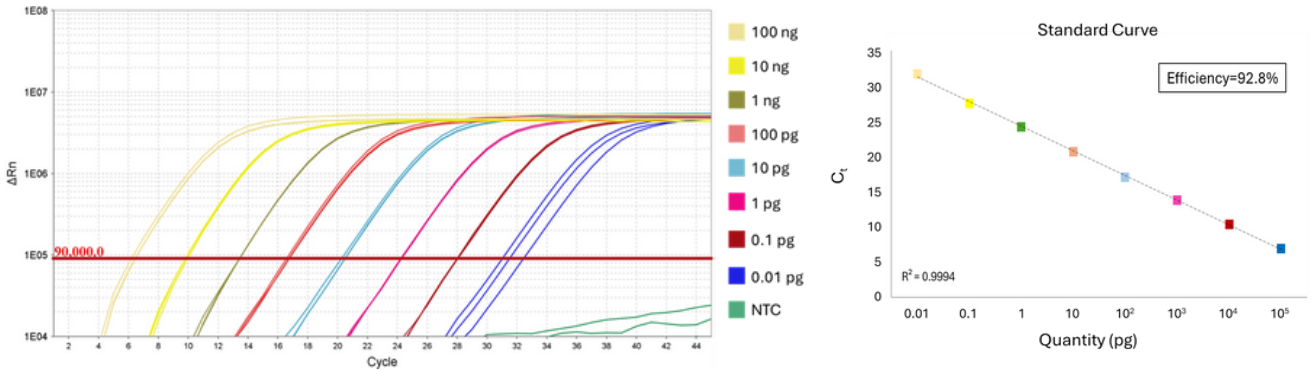

Figure 3. The **UltraMarathonRT Two-Step RT-qPCR Kit** accurately detects RNA inputs from 0.1 pg to > 100 ng with high efficiency and reliability.

UltraMarathonRT Motors Past Troublesome cDNA Synthesis Inhibitors

The ultra-processive UltraMarathonRT enzyme has been engineered to synthesize full-length cDNA from the most difficult, long, highly structured RNA templates, even in the presence of potential enzyme inhibitors. The uMRT RT-qPCR Kit delivers robust, consistent results when faced with a group of inhibitors commonly found in biological samples, including ethanol, isopropanol, TRIzol, formalin, ammonium acetate, and hematin.

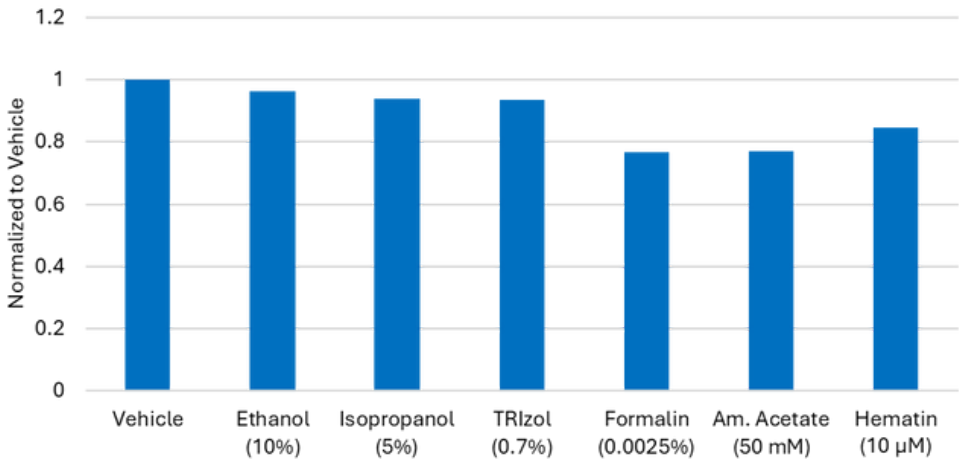

Figure 4. Inhibitors from RNA sample preparation procedures have historically been an issue for some RT-qPCR kits. Here we show that the **UltraMarathonRT Two-Step RT-qPCR Kit** is not impacted by common inhibitors.

Heat Destroys Your RNA Samples

In the past, the only way to unwind RNA secondary structures so that MMLV RTs can get through them was to heat the delicate RNA samples. Each successive generation of MMLV RTs sought higher levels of thermostability to accommodate the excessive temperatures necessary to melt these structures. However, heat rapidly degrades the samples you're interested in. UltraMarathonRT's intrinsic helicase activity unwinds all such secondary structures at ambient temperatures, preserving the integrity of your RNA samples.

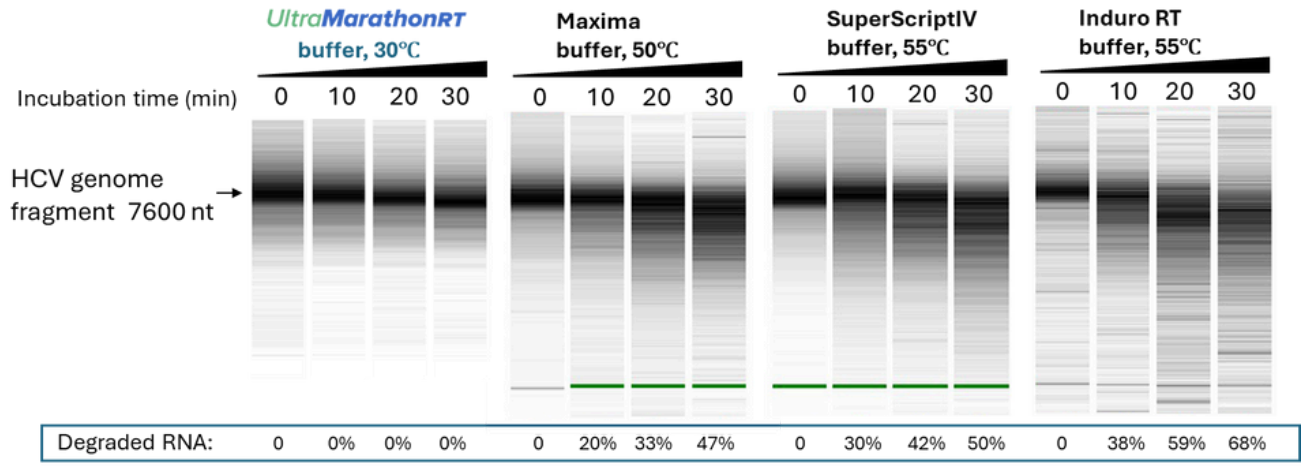

Figure 5. An HCV RNA genome fragment stability was tested by incubating it in the reaction buffers for UltraMarathonRT, Maxima H Minus, SuperScript IV, and Induro at their recommended reaction temperatures for 0, 10, 20, and 30 minutes, respectively. No reverse transcription was performed. The RNA construct showed no perceivable degradation at uMRT operating temperatures, and substantial degradation at the higher working temperatures recommended for the other RTs.

Perform the Two-Step in Less Than an Hour

The **UltraMarathonRT Two-Step RT-qPCR Kit** is designed for flexibility and efficiency, enabling researchers to complete a full two-step RT-qPCR workflow in under one hour. The optimized protocol minimizes reaction time, allowing you to focus on data analysis and discoveries.

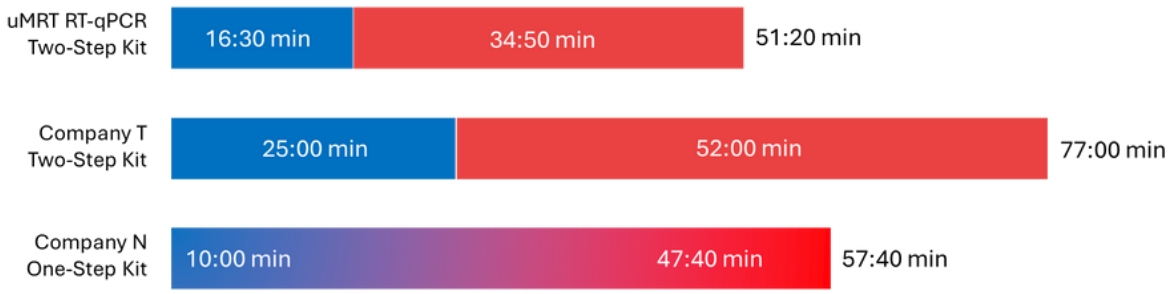

Figure 6. The **UltraMarathonRT Two-Step RT-qPCR Kit** saves time with an overall run time less than one hour.

The **UltraMarathonRT Two-Step RT-qPCR Kit** ensures unmatched data reliability, delivering consistent C_t values regardless of RT primer type, PCR primer location or RNA complexity. Whether working with gene-specific primers, oligo(dT), or random primers, researchers can trust this kit to provide reproducible results. Its robust design eliminates biases caused by variable GC content, stable RNA structures and repeat sequences, ensuring accurate quantification across all templates.

Inhibitory compounds often complicate workflows, but the UltraMarathonRT kit is engineered for high inhibitor tolerance. It maintains exceptional performance in the presence of common inhibitors such as ethanol, isopropanol, TRIzol, formalin, and more. Additionally, its fast reaction and streamlined protocol allows researchers to generate results in under an hour, while dye-based detection facilitates real-time RNA monitoring.

The kit's applications extend beyond standard gene expression analysis. It is ideal for RNA-Seq verification, quantifying small RNAs like miRNAs, analyzing alternative splicing, and assessing RNA degradation or editing. Whether verifying RNAi knockdowns or evaluating CRISPR experiments, this kit provides reliable and precise results, making it a trusted companion for modern RNA studies.

UltraMarathonRT Two-Step RT-qPCR Kit

Kit Components:

- UltraMarathonRT
- 2x RT Reaction Buffer Q
- Nuclease-free Water
- dNTP Mix (10 mM each)
- 2x qPCR Green Dye Master Mix
- Random Primers (15mer) (10 µM)
- Oligo(dT)18 Primer (5 µM)

Catalog Information:

- **R1006S:** 200 reactions
- **R1006M:** 500 reactions
- **R1006L:** 1,000 reactions

For purchase orders or bulk ordering contact us at sales@rnaconnect.com.

SHOP NOW

References

1. Kounosu, A., Murase, K., Yoshida, A., Maruyama, H. & Kikuchi, T. Improved 18S and 28S rDNA primer sets for NGS-based parasite detection. Sci Rep 9, (2019).