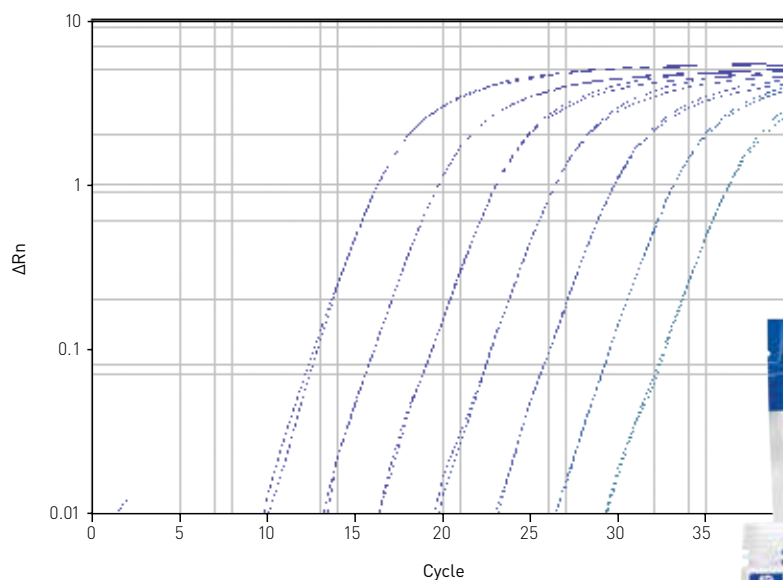


TaqMan® RNA-to-C_T™ 1-Step Kit

Leading-edge sensitivity and PCR efficiency for a wide range of targets

- Accuracy across a wide dynamic range for dependable performance
- High sensitivity, enabling low-copy number detection and precise quantification
- Consistency over a variety of assays including AT-rich, GC-rich, and long amplicons
- Minimized hands-on time with a one-step protocol
- Single-tube reaction with few pipetting steps
- Validated with TaqMan® Gene Expression Assays for easy experimental setup



Introduction

The TaqMan® RNA-to-C_T™ 1-Step Kit is designed to deliver consistent RNA target quantification on a wide variety of assays and is validated with a comprehensive library of TaqMan® Gene Expression Assays from Thermo Fisher Scientific. The easy-to-use one-tube protocol helps minimize cross-contamination compared to a two-step protocol, while the formulation and enzymes provide high sensitivity and quantitative discrimination. The TaqMan® RNA-to-C_T™ 1-Step Kit can be used in a variety of applications, including gene expression studies, pathogen detection, and biomarker discovery and validation.

Formulation

With the TaqMan® RNA-to-C_T™ 1-Step Kit, you can perform reverse transcription and real-time PCR in a single reaction tube. The kit includes:

- AmpliTaq Gold® DNA Polymerase UP (Ultra Pure) for hot-start PCR to prevent amplification during reaction setup
- ArrayScript™ UP reverse transcriptase for high-yield, full-length reverse transcription across a wide range of targets
- A dNTP blend including dUTP, for post-PCR amplicon degradation with uracil-DNA glycosylase (UDG) to minimize carryover contamination in subsequent PCR reactions

- An RNase inhibitor to prevent degradation of RNA template
- A buffer optimized to improve sensitivity across a broad range of targets
- A passive internal reference based on proprietary ROX™ dye, for increased data precision

Efficiency across a broad range of input concentrations

To have maximum flexibility in RNA template input, optimal real-time PCR efficiency is required across a broad range of template quantities. Figure 1 shows the excellent amplification efficiency across 6 orders of magnitude. The results are comparable to those of the TaqMan® RNA-to-C_T™ 2-Step Kit, with significant improvement in dynamic range and sensitivity over TaqMan® One-Step RT-PCR Master Mix reagents.

High sensitivity at low target concentrations

The sensitivity of the TaqMan® RNA-to-C_T™ 1-Step Kit was validated using a synthetic RNA template for which copy number is precisely known. Significant sampling errors occur when measuring low quantities of target, so statistical analysis is required for proper evaluation using multiple replicates. Figure 2 shows the expected quantity of target and corresponding mean C_t values. Statistical analysis indicates high confidence of sample quantification based on a *t*-test (Table 1), consistent with as few as 10 copies of RNA target.

Discrimination between similar abundance levels

Precise quantification of RNA allows researchers to distinguish small differences in gene expression levels. As demonstrated using RNA Control 250, a highly characterized synthetic target used to precisely quantify copy number, the TaqMan® RNA-to-C_T™ 1-Step Kit enables the discrimination of 2-fold and 1.5-fold differences in starting template amounts (Figure 3). The data clearly demonstrate the statistically significant discrimination of the 1.5-fold difference between the RNA samples (1,070 vs. 1,600 copies).

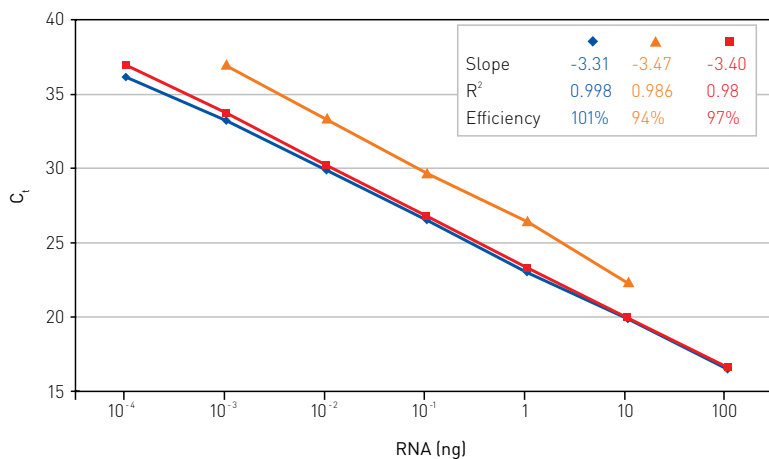


Figure 1. The TaqMan® RNA-to-C_T™ 1-Step Kit (blue) shows a wider dynamic range than TaqMan® One-Step RT-PCR Master Mix reagents (orange), and performance comparable to the TaqMan® RNA-to-C_T™ 2-Step Kit (red). Serial dilutions of PPIA target (containing 100 ng to 0.1 pg RNA) were amplified on the 7900HT Fast Real-Time PCR System.

Table 1. Statistical *t*-test to evaluate detection of small amounts of target shown in Figure 2.

Nominal copies	Mean C _t	<i>t</i> Value	<i>P</i> Value	Confidence
10	35.48	3.34	<0.001	99.9%
20	34.55			
20	34.55	3.34	<0.001	99.9%
30	33.88			
30	33.88	3.34	<0.001	99.9%
40	33.51			
40	33.51	3.34	<0.001	99.9%
50	33.15			
50	33.15	3.34	<0.001	99.9%
100	32.00			

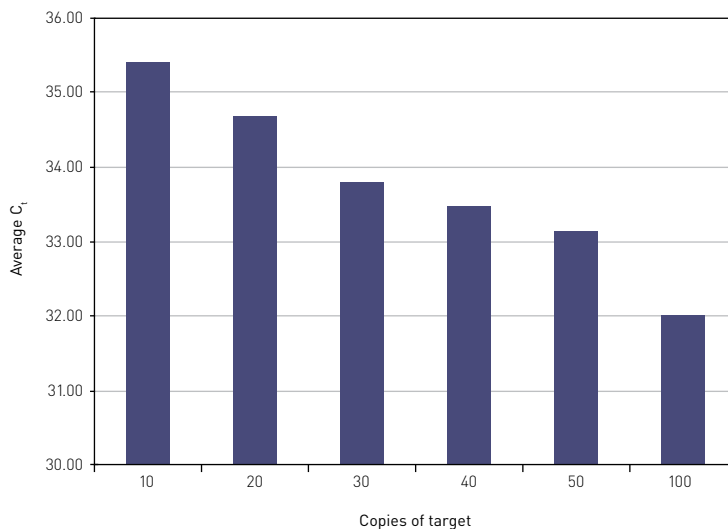


Figure 2. Mean C_t values obtained from amplification of 10 to 100 copies of RNA Control 250 (in 32 replicates) using the TaqMan® RNA-to-C_T™ 1-Step Kit and the 7900HT Fast Real-Time PCR System.

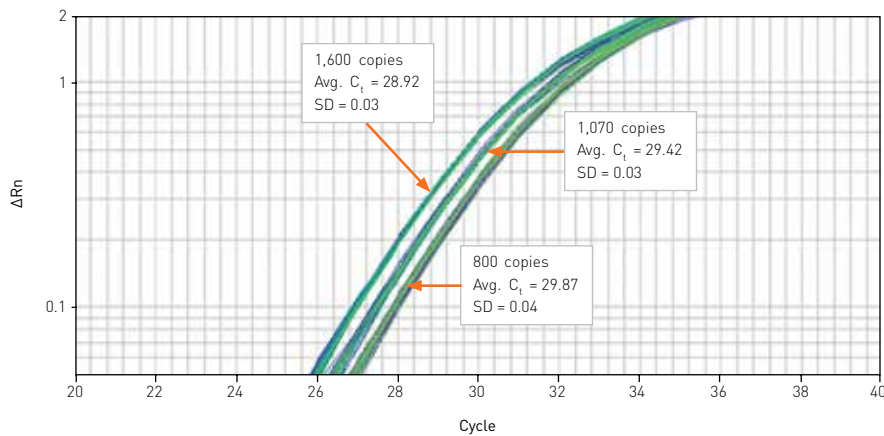


Figure 3. Discrimination among 1,600, 1,070, and 800 copies of starting RNA. A dilution series of RNA Control 250 was prepared: 1,070 copies = 1.5-fold dilution, 800 copies = 2-fold dilution. Replicate reactions (48) were run on the 7900HT Fast Real-Time PCR System.

Robust amplification even with challenging targets

A general-purpose one-step kit must function effectively across a variety of targets, including those with secondary structure or with difficult design constraints. As shown in Figure 4, the TaqMan® RNA-to-C_T[™] 1-Step Kit shows excellent efficiency even for AT-rich and long amplicon targets (>150 bp). Similarly, Figure 5 shows the results of an assay on a GC-rich target comparing the TaqMan® RNA-to-C_T[™] 1-Step Kit to a one-step qRT-PCR kit from another vendor. The TaqMan® RNA-to-C_T[™] 1-Step Kit clearly demonstrates superior real-time PCR performance over the other vendor's kit in efficiency and robustness.

Conclusion

The TaqMan® RNA-to-C_T[™] 1-Step Kit is designed to accommodate the needs of one-step real-time RT-PCR users for a variety of applications. It provides leading-edge sensitivity and PCR efficiency across a wide range of targets, including challenging ones. The single-tube protocol provides ease of use with reduced potential for crossover contamination and error, and can be easily integrated into an automated workflow.

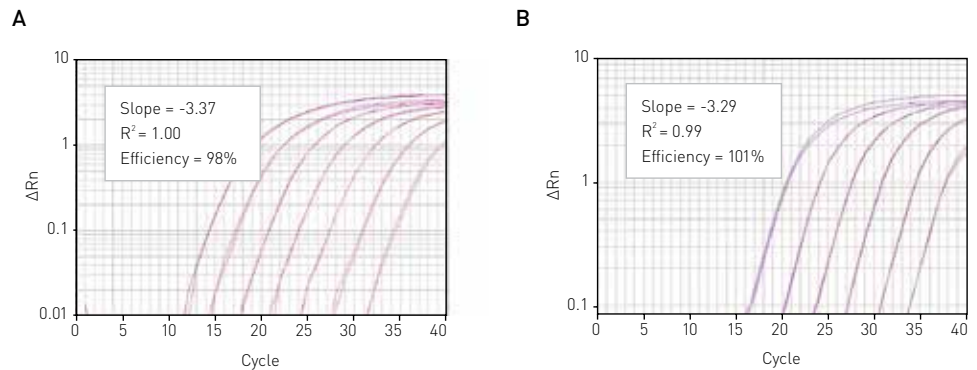


Figure 4. Amplification of (A) RPL18 (200 bp amplicon) and (B) CCT7 (72% AT). Amplifications were from serial dilutions of the targets, using the TaqMan® RNA-to-C_T[™] 1-Step Kit on the 7900HT Fast Real-Time PCR System.

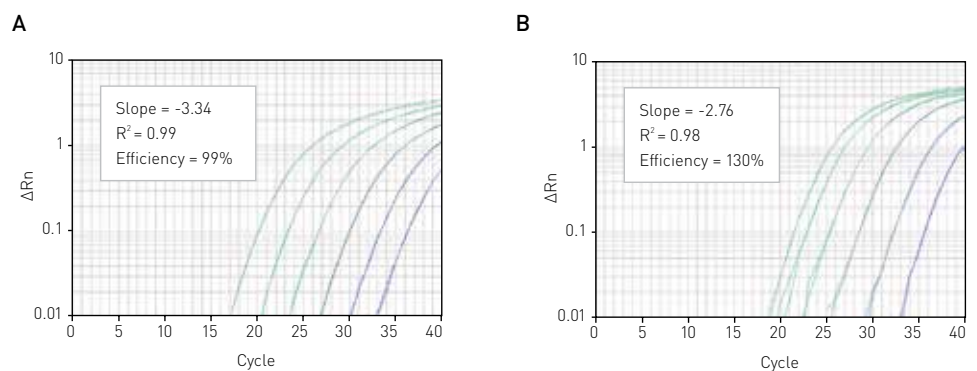


Figure 5. A comparison of the TaqMan® RNA-to-C_T[™] 1-Step Kit with a one-step kit from Vendor Q amplifying a 67% GC region FZD1. The TaqMan® RNA-to-C_T[™] 1-Step Kit (A) shows superior PCR efficiency and, unlike Vendor Q's kit (B), no reaction inhibition at high template amounts. Amplifications were from serial dilutions of the target on the 7900HT Fast Real-Time PCR System.

Ordering information

Description	Quantity	Cat. No.
TaqMan® RNA-to-C _T ™ 1-Step Kit		
Mini-Pack	1 mL tube (40 rxns)*	4392653
1-Pack	5 mL bottle (200 rxns)*	4392938
10-Pack	10 x 5 mL bottle (2,000 rxns)*	4392656
Quick Reference Card	1	4393464
Protocol	1	4393463

* Assumes 50 µL reaction volume.

Find out more at lifetechnologies.com

life
technologies

A Thermo Fisher Scientific Brand