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Thermo Scientific UltraSense One-Step Quantitative RT-PCR System

For amplification of RNA viruses and ultra-low abundance transcripts

ThermoFisher SCIENTIFIC

Ultra-sensitive real-time RT-PCR

The Thermo Scientific™ RNA UltraSense™ One-Step Quantitative RT-PCR System is specially designed for amplification and real-time detection of RNA viruses and ultra-low abundance transcripts. The optimized, ultra-concentrated system combines Superscript™ III Reverse Transcriptase (RT) and Platinum® Taq DNA Polymerase, providing greater priming specificity, higher product yields, and detection over a broad dynamic range.

Optimized enzyme blend for superior performance

The RNA Ultrasense System combines two industry-leading enzymes.

Superscript III Reverse Transcriptase (RT) is a point mutant of Superscript™ II RT with reduced RNase H activity. It exhibits a longer half-life (220 min. at 50 °C) and increased thermostability, providing higher cDNA yields, greater success with RNA secondary structure, and increased specificity with gene-specific primers.

Platinum® Taq DNA Polymerase provides antibody-mediated hot-start technology to reduce mispriming and nonspecific amplification, resulting in greater specificity and sensitivity at the PCR step.

By incorporating Superscript III RT and Platinum Taq DNA Polymerase, the RNA UltraSense System offers the most sensitive, specific one-step system available for real-time qRT-PCR.

Ultra-concentrated system for sensitive amplification of low-abundance RNA

The RNA UltraSense System is particularly useful for the study of rare transcripts or for amplification of dilute templates. The 5X qRT-PCR reaction mix permits ~70% of the reaction mixture volume (Table 1) to be your sample-2.5X more concentrated than other quantitative RT-PCR systems. This higher concentration formulation provides greater flexibility with low concentration/high volume RNA samples. As little as 25 pg/µL total RNA can be detected with a PCR efficiency of 97% (Figure 1).

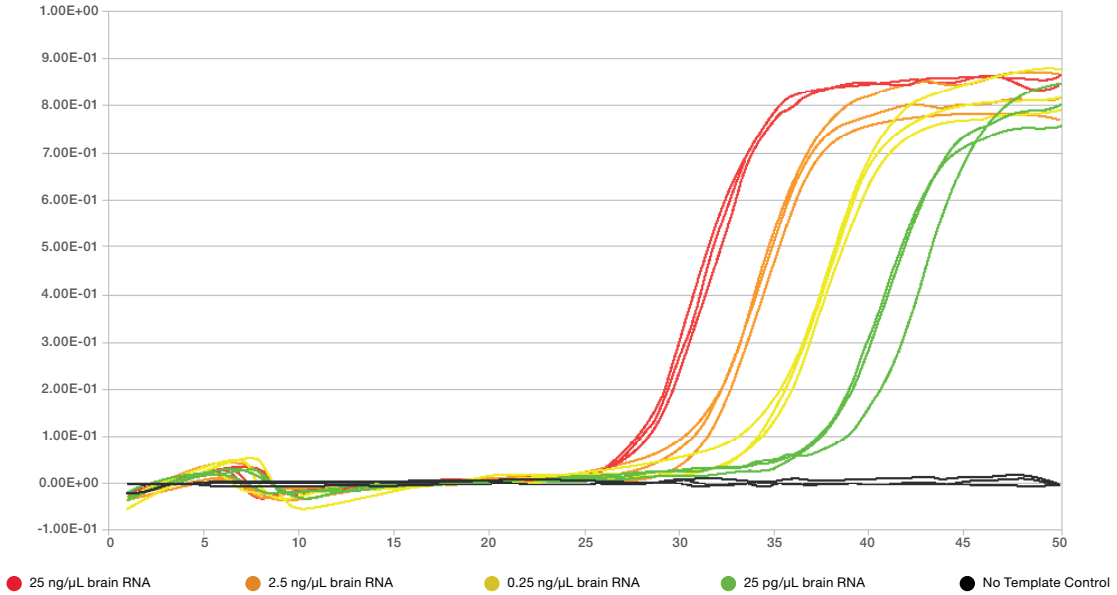
Table 1 - Quantitative RT-PCR with the RNA UltraSense System allows greater sample volumes

RNA UltraSense System with LUX™ Fluorogenic Primers:			
Component	**20 µL reaction volume**	**50 µL reaction volume**	**Final concentration**
RNA UltraSense™ enzyme mix	1 µL	2.5 µL	1X
RNA Ultrasense™ reaction mix (5X)	4 µL	10 µL	1X
LUX™ labeled forward primer (10 mM)	0.4 µL	1 µL	200 nM
Unlabeled reverse primer (10 mM)	0.4 µL	1 µL	200 nM
50X ROX Reference Dye	0.4 µL	1 µL	1X
Template	**up to 14.2 µL**	**up to 35.5 µL**	**71%**

RNA UltraSense System with TaqMan® Probes:			
Component	**20 µL reaction volume**	**50 µL reaction volume**	**Final concentration**
RNA UltraSense™ enzyme mix	1 µL	2.5 µL	1X
RNA Ultrasense™ reaction mix (5 X)	4 µL	10 µL	1X
Unlabeled primer, forward (10 mM)	0.4 µL	1 µL	200 nM
Unlabeled primer, reverse (10 mM)	0.4 µL	1 µL	200 nM
Labeled probe (dye/quencher, 10 mM)	0.4 µL	1 µL	100 nM
50X ROX Reference Dye	0.4 µL	1 µL	1X
Template	**up to 13.4 µL**	**up to 33.5 µL**	**67%**

Figure 1 - High-sensitivity RNA detection with high qRT-PCR efficiency

Amplification of mChAT from Brain RNA



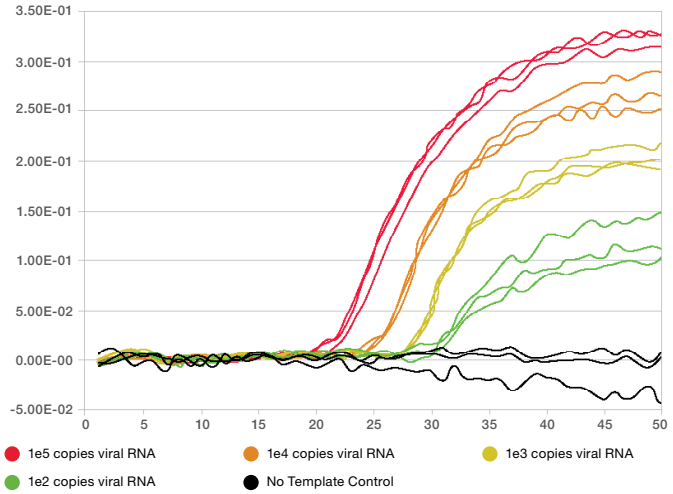
Total RNA from mouse brain was diluted to 25 ng/µL, 2.5 ng/µL, 0.25 ng/µL and 25 pg/µL. Thirteen microliters of each dilution were added to triplicate 20-µL reactions. The RNA UltraSense™ One-Step qRT-PCR System was able to amplify the rare ChAT message from RNA samples as dilute as 25 pg/µL with a PCR efficiency of 97%.

Success even with considerable RNA secondary structure

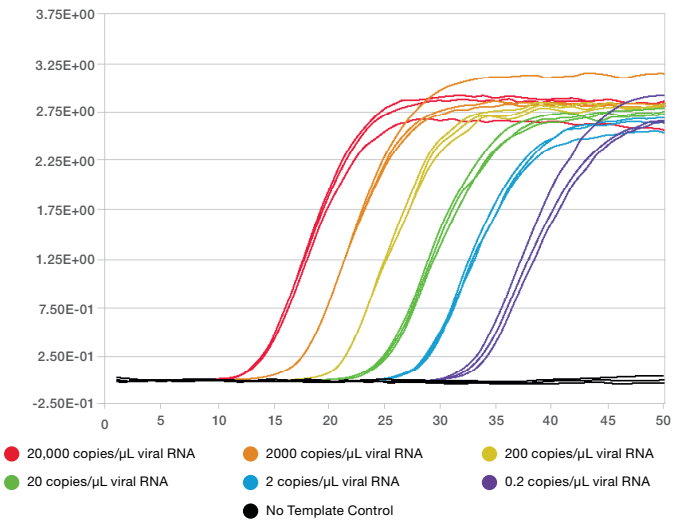
RNA, especially viral RNA, can be especially difficult to amplify due to the presence of significant amounts of secondary structure. The RNA Ultrasense System includes a proprietary enzyme mixture that promotes primer-template interactions under these difficult conditions, improving performance (Figure 2). Additionally, the high thermostability of Superscript III RT-enabling cDNA synthesis at temperatures up to 60°C-increases success with targets rich in RNA secondary structure.

Figure 2 - The RNA UltraSense System provides improved amplification of viral RNA with significant secondary structure

A Amplification of APMV-1 Vaccine RNA



B Amplification of pLenti6V5GFP viral RNA



The Newcastle virus is a single-stranded RNA virus that exhibits considerable secondary structure. Real-time qRT-PCR was performed on 10^5, 10^4, 10^3, 10^2, or 10 copies of END RNA using Taqlvlan® primers and probe against viral sequence. Immediately before loading, the templates were heated to 95°C for 5 minutes and subsequently placed on ice.

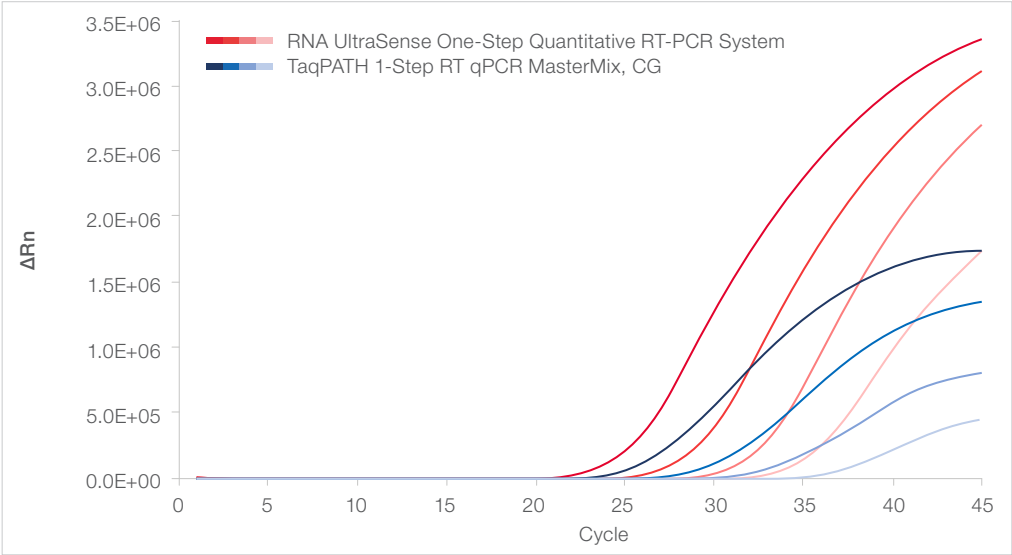
(A) RNA Ultrasense System with SuperScript III RT provides clean amplification of four serial dilutions of RNA with exceptional efficiency (107%). A standard, competing one-step RT-PCR system exhibited poor performance when encountering significant secondary structure. The system was only able to amplify two serial dilutions of RNA template.

(B) Amplification of 6 serial dilutions of pLenti6V5GFP viral RNA, efficiency of 90%.

MasterMix comparison study for detection of SARS-CoV-2

During the COVID-19 pandemic food and beverage companies, retailers and public health authorities seek solutions to monitor the presence of SARs-CoV-2 in their environments. As part of the response to address this need a study was conducted to determine the suitability of the TaqMan™ 2019-nCoV Assay Kit v1 for detecting SARs-CoV-2 in environmental samples, including a comparison between Master Mixes. The data in Figure 3 shows the assay results when using RNA UltraSense One-Step Quantitative RT-PCR System, as commonly used with environmental samples, and TaqPath 1-Step RT-qPCR Master Mix.

Figure 3 – Earlier and stronger fluorescence signal for 2019-nCoV N protein when using RNA UltraSense



Target 2019-nCoV N protein using primers and probes in the TaqMan™ 2019-nCoV Assay Kit v1

RNA input per reaction using the 2019-nCoV Control Kit v1: 10,000 copies, 1,000 copies, 100 copies and 10 copies. Cycling parameters according to TaqMan™ 2019-nCoV Assay Kit v1.

Ordering information

Product	Format	Product code
Thermo Scientific™ Ultrasense™ One-Step Quantitative RT-PCR System	100 reactions	11732927

Find out more at **thermofisher.com/ultrasense**

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LT2546A
Updated January 2021

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