

## Reverse transcription

## SuperScript V Reverse Transcriptase

## Introduction

Invitrogen™ SuperScript™ V Reverse Transcriptase represents the next generation of cDNA synthesis technology, developed to meet the growing demand for advanced RNA analysis workflows where sensitivity, reliability, and efficiency are critical. As applications such as single-cell and low-input RNA sequencing, long-read transcriptomics, and rare target detection continue to push technical boundaries, researchers increasingly face challenges with limited, degraded, or inhibitor-rich samples. SuperScript V Reverse Transcriptase has been engineered to overcome these barriers, helping deliver high-quality cDNA with exceptional sensitivity and robustness across diverse sample types—from formalin-fixed, paraffin-embedded (FFPE) tissue to low-input and partially degraded RNA samples.

In addition, it introduces meaningful workflow innovations, including rapid reaction times, room temperature stability, and ambient setup capability, enabling greater flexibility and control at the bench. Combined with a formulation designed to reduce environmental impact, SuperScript V Reverse Transcriptase empowers scientists to generate reliable results while improving efficiency and supporting more sustainable lab practices.

SuperScript V Reverse Transcriptase is available in multiple formats to support a wide range of workflows and user preferences:

- **SuperScript V Reverse Transcriptase**—maximum flexibility to customize reaction components and optimize protocols for specialized applications
- **Invitrogen™ SuperScript™ V VILO™ Master Mix**—convenient, ready-to-use format with optimized components for fast setup and consistent, high-performance results in reverse transcription quantitative PCR (RT-qPCR)
- **Invitrogen™ SuperScript™ V VILO™ Master Mix, Primer-Free**—greater control over priming strategy, enabling tailored applications and compatibility with diverse downstream workflows

The following experiments evaluate the performance of SuperScript V Reverse Transcriptase and SuperScript V VILO Master Mix across a range of RNA inputs, sample conditions, and workflow requirements.



## Key highlights

- **Exceptional sensitivity**—broad input range from 100 fg to 5 µg
- **Robust performance**—confidently process challenging samples
- **Faster workflows**—generate high-quality cDNA in as little as 11 minutes with streamlined protocols and fewer steps
- **Simplified logistics**—ambient shipping conditions and room temperature stability for up to 6 months
- **Flexible setup**—helper protein enables room temperature setup and stability of assembled reactions for up to 24 hours
- **Sustainable design**—animal origin-free (AOF) and DTT-free formulation with reduced cold chain requirements

## Materials and methods

### Commercial RNA samples

Commercial RNA samples used included Invitrogen™ Cervical Adenocarcinoma (HeLa-S3) Total RNA (Cat. No. AM7852), Invitrogen™ Millennium™ RNA Markers (Cat. No. AM7150), and Control GAPDH RNA (component from the Thermo Scientific™ RevertAid™ First Strand cDNA Synthesis Kit, Cat. No. K1621).

### Purification of RNA

Total RNA was isolated from mouse heart (~0.1 g) using RNAzol™ RT reagent (Molecular Research Center, Cat. No. RN190) according to the manufacturer's protocol. Tissues were homogenized with RNAzol RT reagent, followed by addition of nuclease-free water to precipitate DNA, proteins, and polysaccharides. Following centrifugation, RNA in the supernatant was precipitated with isopropanol, pelleted by centrifugation, washed twice with 75% ethanol, air-dried, and dissolved in RNase-free water.

### Generation of degraded RNA

1 µg of Invitrogen™ Cervical Adenocarcinoma (HeLa-S3) Total RNA (Cat. No. AM7852) was degraded to an RNA integrity number (RIN) of 3 by adding MgCl<sub>2</sub> to a final concentration of 1 mM and incubating at 95°C for 10 minutes. After incubation, RNA was purified using the Thermo Scientific™ GeneJET™ RNA Cleanup and Concentration Micro Kit (Cat. No. K0841) according to the accompanying protocol. Purified RNA was quantified using a Thermo Scientific™ NanoDrop™ instrument. RNA quality was assessed using the Agilent™ 2100 Bioanalyzer™ system and Agilent™ RNA 6000 Nano Kit.

### cDNA synthesis protocol

Reverse transcription reactions were prepared according to the respective user manuals and performed using the suggested cDNA synthesis conditions unless otherwise specified. Oligo (dT)<sub>18-20</sub> primers, random hexamer primers, or a mix of both were used for cDNA synthesis.

### cDNA synthesis using Millennium RNA Markers

Reverse transcription reactions for all tested reverse transcription products were set up according to the manufacturers' protocols using oligo(dT)<sub>18</sub> primers and 1 µg of Millennium RNA Markers. cDNA synthesis reactions were carried out for 10 min at varying temperatures (37°C–65°C). Enzyme inactivation was performed for 5 minutes at 85°C. The resulting cDNA was analyzed by gel electrophoresis using 2 µL of sample per well.

### cDNA synthesis for evaluation of benchtop stability

Reverse transcription reactions were set up according to the user manual. Upon assembly, one set of reactions was immediately transferred to a thermal cycler for reverse transcription. The other set of reactions was left at room temperature (~22°C) for 4–24 hours before running reverse transcription reactions. cDNA samples were analyzed by qPCR or endpoint PCR.

### qPCR

qPCR was performed on the reverse transcription samples using Applied Biosystems™ PowerTrack™ SYBR™ Green Master Mix (Cat. No. A46012) or TaqMan™ Fast Advanced Master Mix (Cat. No. 4444556) with Applied Biosystems™ TaqMan™ Assays targeting the genes indicated in the figures. qPCR reactions were performed in either 20 µL volumes in Applied Biosystems™ MicroAmp™ Fast Optical 96-Well Reaction Plates (Cat. No. 4346907) or 10 µL volumes in Thermo Scientific™ 384-Well PCR Plates (Cat. No. AB1384). cDNA samples made up to 10% of the total qPCR reaction volume. Assays were performed on an Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System, QuantStudio™ 6 Flex Real-Time PCR System, or QuantStudio™ 7 Flex Real-Time PCR System.

### Endpoint PCR

PCR amplification was performed on an Applied Biosystems™ ProFlex™ PCR System with Invitrogen™ Platinum™ SuperFi™ II Green PCR Master Mix (Cat. No. 12369010) following the protocol in the user guide. Up to 8 µL of each PCR reaction was resolved by agarose gel electrophoresis in TAE buffer and visualized by ethidium bromide staining.

### ezDNase Master Mix treatment

To determine the efficiency of gDNA reduction, 9 µL of an RNA/DNA mixture containing 1,000 ng HeLa total RNA and 50 ng human gDNA (purified from human blood using the Invitrogen™ PureLink™ Genomic DNA Mini Kit (Cat. No. K182001)) was mixed with 1 µL of 10X Invitrogen™ ezDNase™ Master Mix and incubated for 2 min at 37°C in 10 µL reactions. After ezDNase Master Mix treatment, samples were evaluated in qPCR and compared to equivalent samples without the gDNA reduction step.

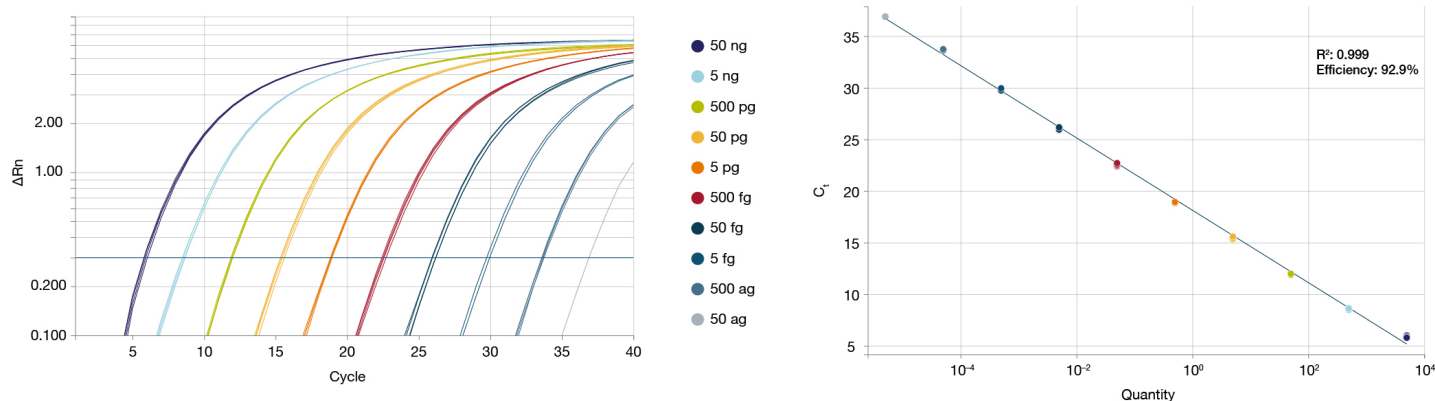
To evaluate the effect of ezDNase Master Mix on RNA integrity in RT-qPCR, 10 pg–1 µg of HeLa total RNA was pretreated with ezDNase Master Mix, followed by reverse transcription, and compared to samples where ezDNase Master Mix was not used. Samples were evaluated by comparing C<sub>t</sub> values, R<sup>2</sup> values, and amplification efficiency.

## Results and discussion

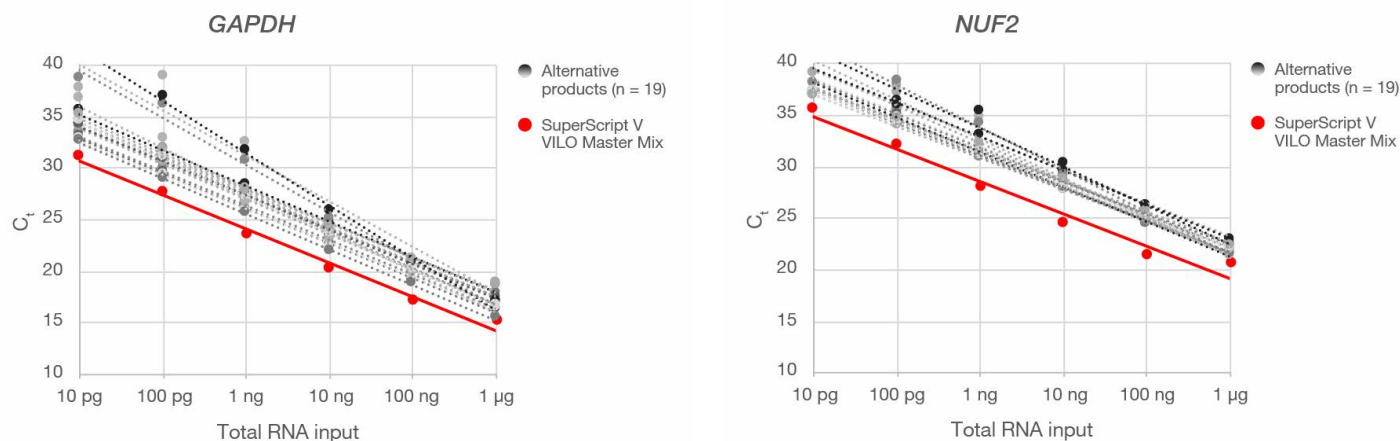
### Superb RT-qPCR performance starts with high-quality cDNA

SuperScript V VILO Master Mix supports RT-qPCR workflows with exceptional sensitivity, allowing consistently earlier  $C_t$  values that reflect higher cDNA yields and enabling reliable detection of low-abundance targets. The master mix also helps ensure accurate and reproducible quantification across a broad range of input RNA over multiple orders of magnitude (Figure 1). Compared to reverse transcription products from other suppliers, SuperScript V VILO

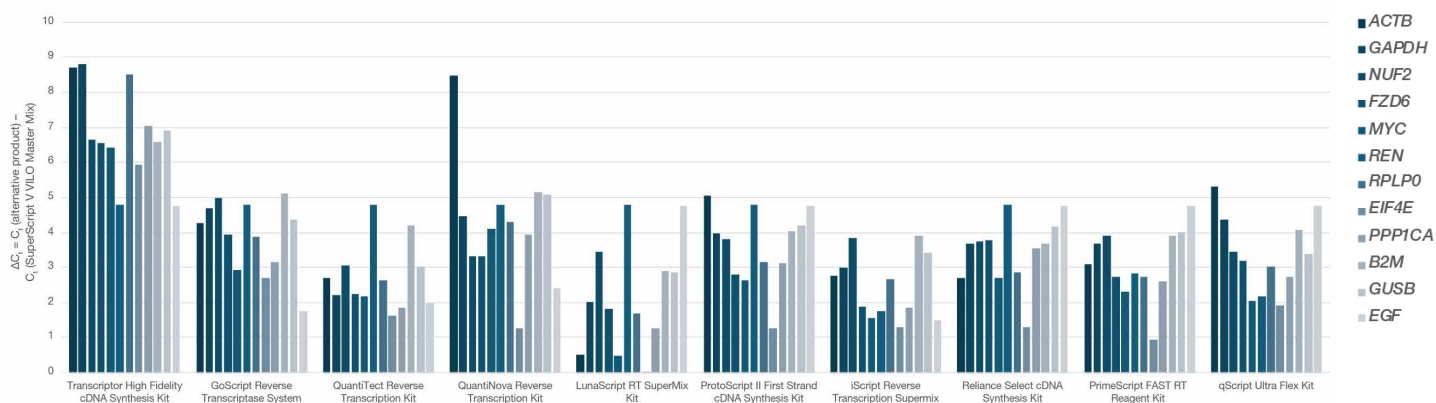
Master Mix shows a clear performance advantage, with alternative products exhibiting  $C_t$  delays of up to 8 cycles (Figures 2 and 3). Together, these results highlight superb sensitivity, consistency, and dynamic range, making SuperScript V VILO Master Mix an excellent choice for applications involving demanding gene expression analysis.



**Figure 1. Ultra-broad dynamic range and sensitive detection in RT-qPCR.** Distinct, well-separated amplification curves demonstrate consistent performance across multiple orders of magnitude, supporting excellent linearity ( $R^2 = 0.999$ ) and high amplification efficiency (92.9%).



**Figure 2. Early  $C_t$  values indicate excellent sensitivity and cDNA yield.** Compared to 19 other commercial reverse transcription products, SuperScript V VILO Master Mix consistently achieves earlier  $C_t$  values (red line) across a broad range of input RNA, reflecting higher cDNA yield and enhanced detection sensitivity. All products were evaluated using manufacturer-provided protocols.



**Figure 3. Up to 8 cycles earlier detection with SuperScript V VILO Master Mix.** Across multiple tested gene targets, alternative reverse transcription products showed positive  $\Delta C_t$  values of up to ~8 cycles relative to SuperScript V VILO Master Mix, indicating later target detection.

Robust performance across sample types

SuperScript V VILO Master Mix supports reliable performance across a wide range of challenging sample types by exhibiting high sensitivity and robust activity under conditions that commonly inhibit reverse transcription. It consistently supports early  $C_t$  values even in the presence of inhibitors such as humic acid, heparin, ethanol, salts, and detergents, where other commercial products show delayed or undetectable signals (Figure 4). Reliable performance is also maintained with degraded RNA (Figure 5), supporting accurate

detection when sample quality is compromised. In addition, its high thermostability supports efficient cDNA synthesis across a wide temperature range, including elevated temperatures that help resolve RNA secondary structure—enabling effective transcription of difficult, GC-rich templates and long fragments (Figures 6 and 7). Together, these capabilities support dependable, high-quality results across a broad spectrum of real-world sample conditions.

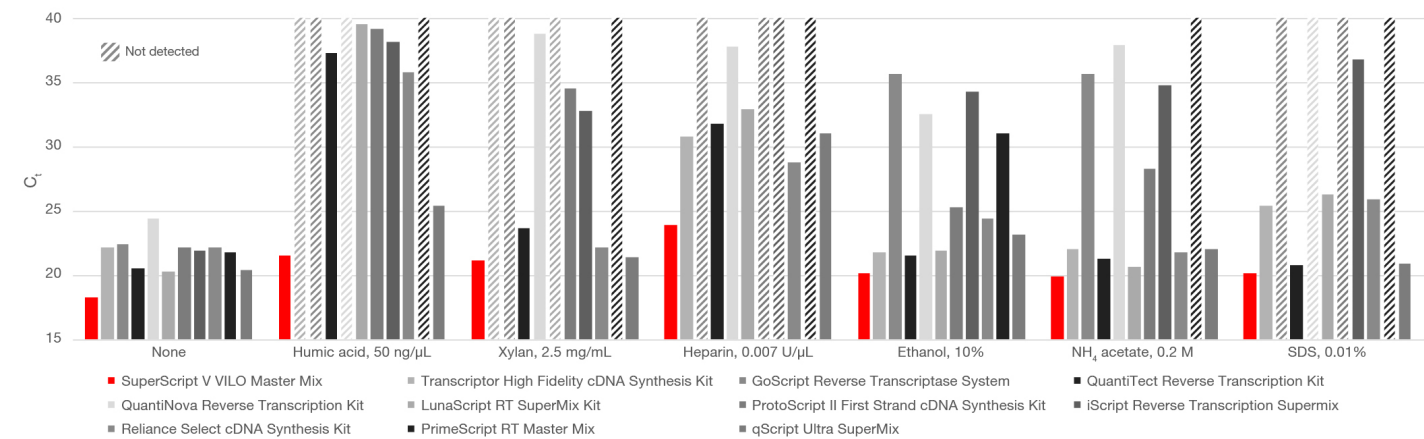


Figure 4. Robust performance in the presence of common inhibitors of reverse transcription. SuperScript V VILO Master Mix consistently shows the earliest  $C_t$  values across all tested conditions, demonstrating strong resistance to inhibition.

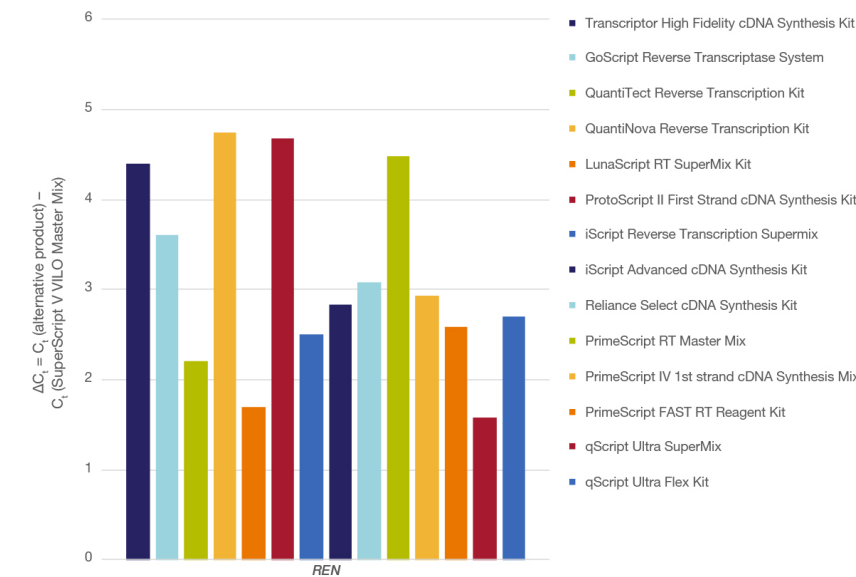


Figure 5. Earlier detection with degraded RNA sample. For *REN* target, SuperScript V VILO Master Mix showed a  $\Delta C_t$  of up to ~4.7 cycles with degraded RNA input ( $\text{RIN} = 3$ ), indicating substantially earlier detection than the alternative products.

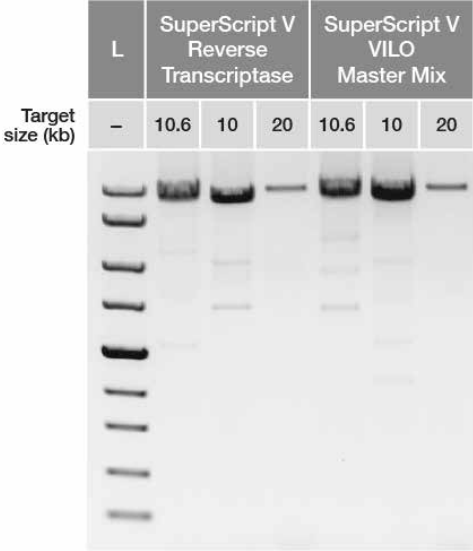
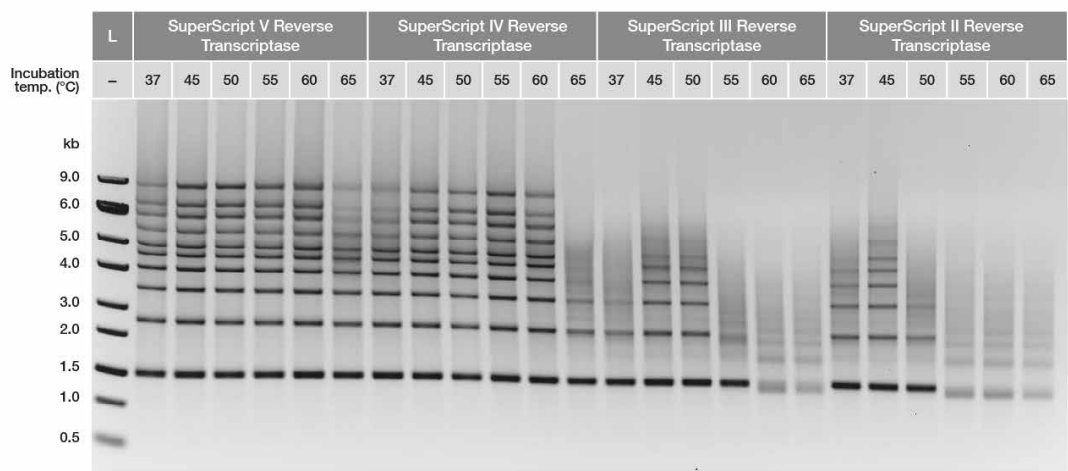


Figure 6. Long cDNA synthesis up to 20 kb. Agarose gel analysis of long-range RT-PCR products generated from mouse heart RNA (1  $\mu$ g per reaction) using SuperScript V Reverse Transcriptase and SuperScript V VILO Master Mix. SuperScript V products enable efficient synthesis and amplification of full-length cDNA approaching 20 kb, supporting robust performance with long RNA transcripts.



**Figure 7. SuperScript V Reverse Transcriptase efficiently transcribes at elevated reaction temperatures up to 65°C.** Agarose gel analysis of cDNA synthesized from Millennium RNA Markers (0.5–9 kb) using all generations of SuperScript enzymes. SuperScript V Reverse Transcriptase efficiently synthesizes long cDNA fragments up to 9 kb across all tested temperatures.

### Fast, streamlined workflow

SuperScript V VILO Master Mix accelerates RT-qPCR workflows by combining speed, simplicity, and high performance in a streamlined format. Integrated ezDNase Master Mix treatment allows efficient gDNA reduction in just 2 minutes, followed by direct addition of the reverse transcription mix in a single-tube workflow—reducing cleanup steps, hands-on time, and sample loss or contamination risk (Figures 8 and 9). Importantly, this workflow simplification does not compromise data quality, preserving RNA integrity and enabling robust, reproducible results (Figure 10).

#### Traditional reverse transcriptase workflow with DNase I (105 min)



#### SuperScript IV VILO Master Mix workflow with ezDNase Enzyme (27 min)

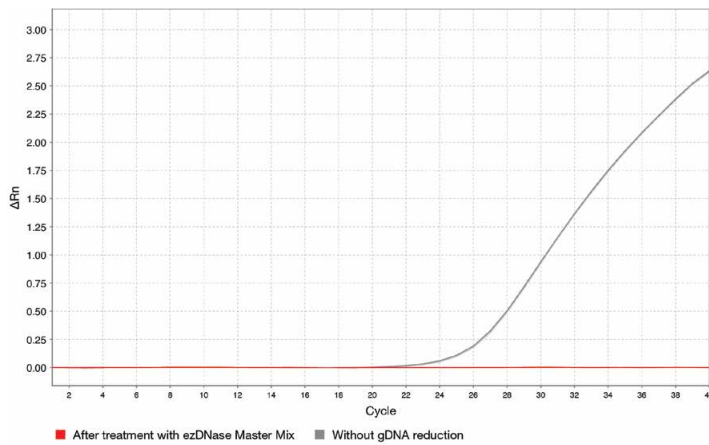


#### SuperScript V VILO Master Mix workflow with ezDNase Master Mix (13 min)

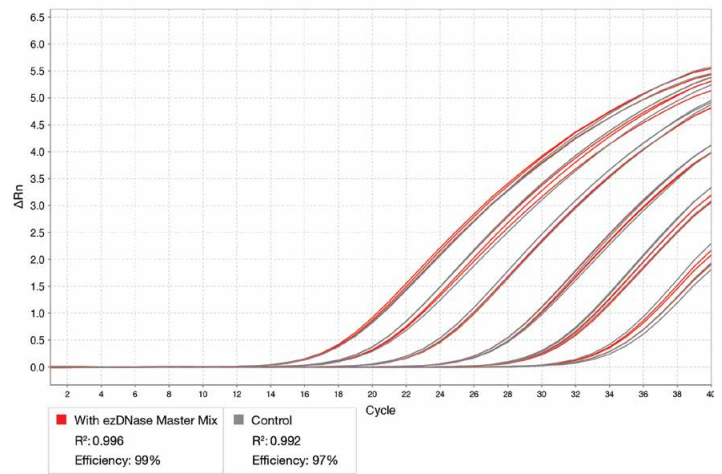


- DNase treatment
- DNase inactivation
- Primer annealing
- Reverse transcription reaction
- Reverse transcription inactivation

**Figure 8. Streamlined workflow with shorter reverse transcription time and simplified processing.** Compared to traditional reverse transcription with DNase I treatment, the SuperScript V VILO Master Mix workflow with ezDNase Master Mix reduces total workflow time through shorter incubations and further streamlines processing by eliminating primer annealing and reducing heat inactivation to 1 minute.



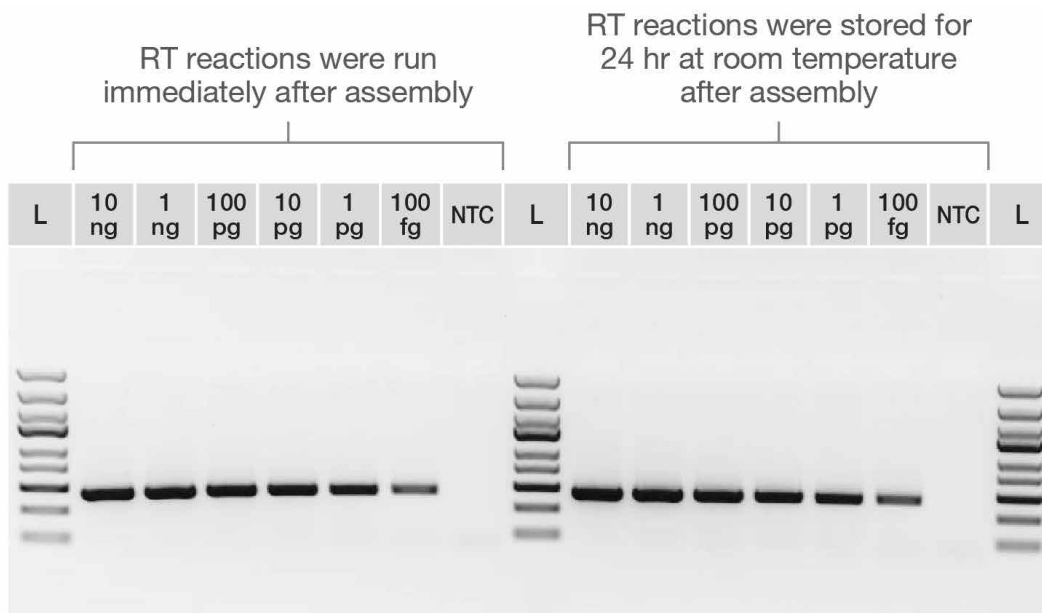
**Figure 9. Rapid and effective gDNA removal in 2 minutes.** ezDNase Master Mix enables efficient gDNA removal, with no detectable amplification signal after treatment compared with strong signal in untreated samples.



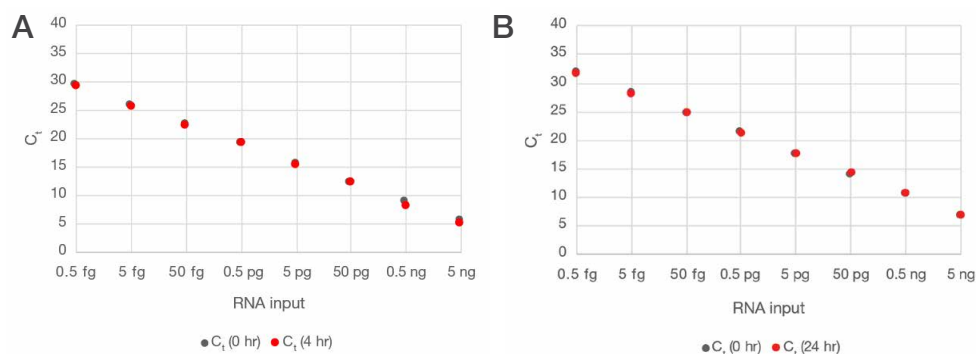
**Figure 10. ezDNase Master Mix treatment preserves RNA integrity in a one-tube workflow.** RT-qPCR analysis of the *ACTB* target shows comparable  $C_t$  values, amplification curves, and linearity between ezDNase Master Mix-treated and untreated samples.

### Reaction setup that fits your schedule

SuperScript V Reverse Transcriptase and SuperScript V VILO Master Mix offer the flexibility to set up reactions on your schedule by incorporating a helper protein that suppresses enzymatic activity at room temperature. This enables convenient benchtop reaction assembly without the need for immediate thermal cycling, reducing time pressure and simplifying workflow planning. Assembled reactions remain stable for up to 24 hours at room temperature (Figures 11 and 12), allowing batching, automation, or delayed runs without compromising performance. This added flexibility supports more efficient lab workflows while maintaining consistent, high-quality results.



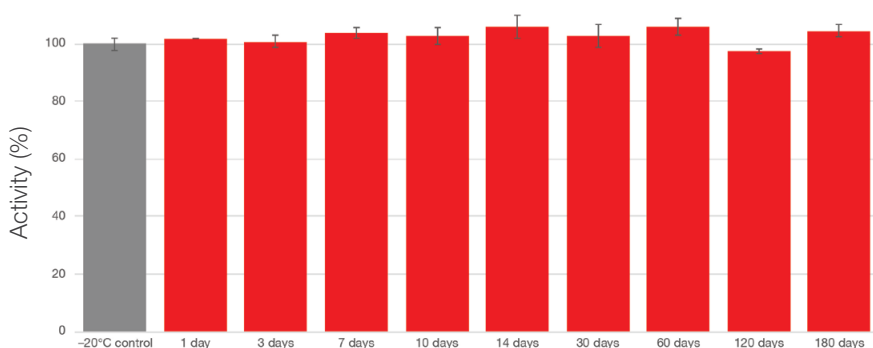
**Figure 11. 24-hour benchtop stability of assembled reactions.** Agarose gel analysis shows consistent cDNA yield across RNA inputs (10 ng to 100 fg) for reactions run immediately after assembly or after 24 hours at room temperature (22°C), demonstrating robust performance after extended benchtop incubation.



**Figure 12. Stable RT-qPCR performance after room temperature reaction setup.** Comparable  $C_t$  values were observed across RNA inputs when reactions assembled with SuperScript V VILO Master Mix were held for up to 24 hours at room temperature. qPCR assays used either (A) Applied Biosystems™ SYBR™ Green dye or (B) TaqMan primers and probe for detection.

### Simplified handling and storage

SuperScript V Reverse Transcriptase simplifies handling and storage through an advanced *in silico*-designed protein that enhances enzyme stability. This supports ambient shipping without the need for cold-chain logistics and enables room temperature storage for up to 6 months for stand-alone reverse transcriptase and up to 1 month for SuperScript V VILO Master Mix formats (Figure 13). These features reduce storage constraints, streamline logistics, and permit added flexibility for everyday use.



**Figure 13. Enzyme activity after storage at room temperature for an extended time.** Enzyme activity of SuperScript V Reverse Transcriptase stored at room temperature (25°C) for 6 months was comparable to that of enzyme stored at -20°C.

### Conclusion

SuperScript V Reverse Transcriptase supports sensitive and robust cDNA synthesis across a broad range of RNA inputs and challenging sample conditions. Efficient cDNA synthesis is observed with common reverse transcription inhibitors, degraded RNA, and long RNA transcripts, including cDNA approaching 20 kb. It also supports streamlined RT-qPCR workflows through integrated ezDNase Master Mix treatment and rapid reverse transcription while maintaining RNA integrity and consistent performance. Assembled reactions remain stable at room temperature for up to 24 hours, supporting flexible reaction setup. Room temperature storage studies further demonstrate consistent cDNA synthesis after extended storage, supporting simplified handling and logistics. Together, these results demonstrate the flexibility, sensitivity, and robustness of SuperScript V Reverse Transcriptase for diverse RNA analysis workflows.

Learn more at [thermofisher.com/superscript](https://thermofisher.com/superscript)

**invitrogen**