SuperScript® IV Reverse Transcriptase: A New Reverse Transcriptase for RNA Analysis

invitrogen by Thermo Fisher Scientific

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ABSTRACT

Survey and interview studies conducted over a three year period revealed that researchers are not satisfied with their current reverse transcriptase and are performing reactions with increasingly difficult samples, such as poorly purified RNA and unpurified RNA (direct RT) that both contain inhibitors. To meet this performance gap, the Thermo Fisher Life Sciences Solutions group produced a new reverse transcriptase, SuperScript® IV, and experiments we performed show that it is the most robust reverse transcriptase compared to other enzymes. SuperScript® IV characterization was performed in the context of "real world" situations where users do not have perfect RNA samples. In the presence of a variety of inhibitors, we demonstrate that SuperScript® IV possesses superior performance in a variety of inhibitors, such as alcohols, salts, detergents, phenol, heparin, hematin, bile salts, and formalin typically found in sample preparation reagents, cell lines, blood, feces, and FFPE samples. This enzyme can even detect RNA targets in unpurified RNA samples (directly lysed cells) and whole blood without sacrificing sensitivity and yield. The introduction of SuperScript® IV enables researchers to obtain more consistent results independent of sample quality and simplify and speed up workflows by eliminating RNA purification.

INTRODUCTION

Gene expression starts when RNA is transcribed from DNA. Expression levels of different RNA targets can be used to characterize species, tissue types, cell types, and healthy and diseased cells. Because RNA is unstable, it is necessary to convert these molecules into more stable ones without loss of quantitative and coding information. Thus, reverse transcriptases are indispensable enzymes because they convert RNA into DNA (cDNA), a stable form of the genetic code. SuperScript®IV is the newest member of the SuperScript® family of reverse transcriptases, which is known for its quality and reliability in cDNA synthesis. This new enzyme has been proven to function in experimentally challenging conditions that many scientists face, such as in the presence of enzyme inhibitors found in reagents, cells, blood, feces, and FFPE samples. To demonstrate this enzyme's capabilities even further, RNA transcripts were detected in unpurified RNA samples.

REVERSE TRANSCRIPTION PROTOCOL

SuperScript® IV First-Strand cDNA Synthesis Reaction

Steps	Procedure
1	Anneal primer to template RNA
2	Prepare RT reaction mix
3	Combine annealed RNA and RT reaction mix
4	Incubate reactions
5	Optional: Remove RNA
6	PCR amplification

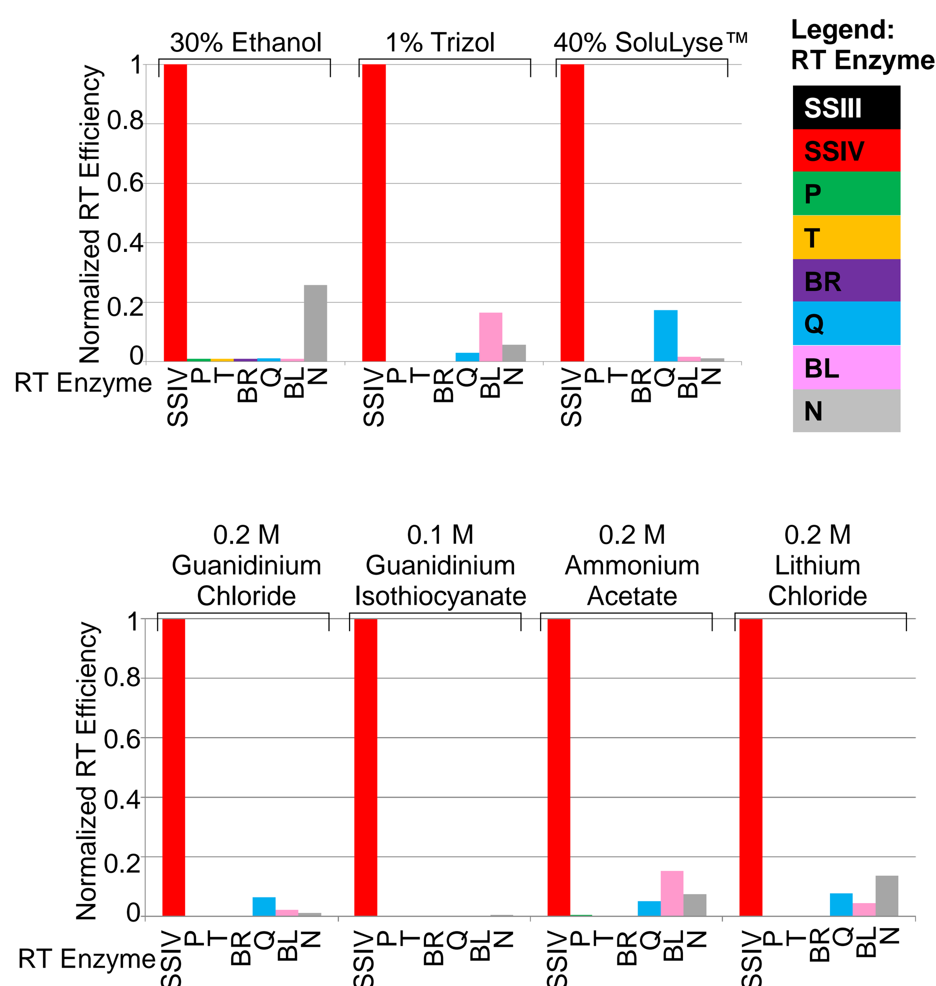
RESULTS

Figure 1. Reverse transcriptase Inhibitors

Inhibitors	Source
Alcohols (Ethanol)	Sample prep
Salts • Guanidinium chloride • Guanidinium isothiocyanate • Ammonium acetate • Lithium chloride	Sample prep
Cell lysis • Trizol • SoluLyse	Sample prep
Heparin, hematin, bile salts	Cells, blood, feces
Formalin	FFPE

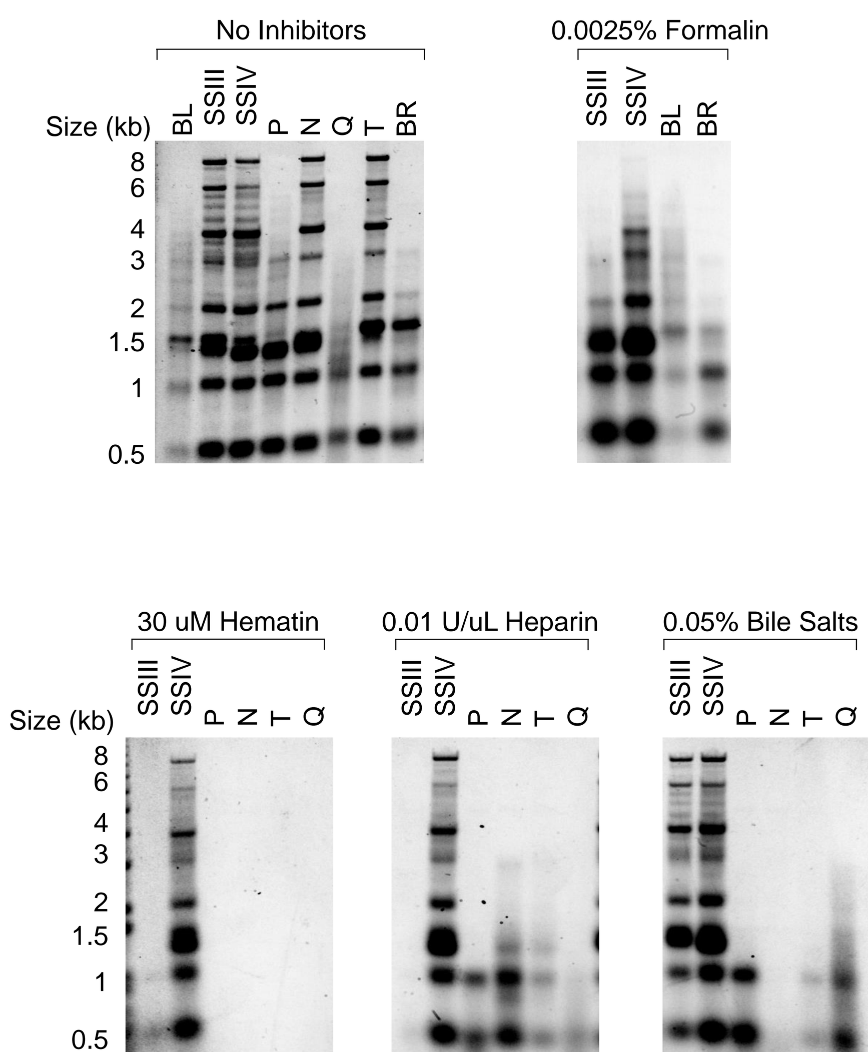
Let no sample go unconquered — Introducing all-new SuperScript® IV RT

Figure 2. SuperScript® IV RT-qPCR Performance in the Presence of Inhibitors



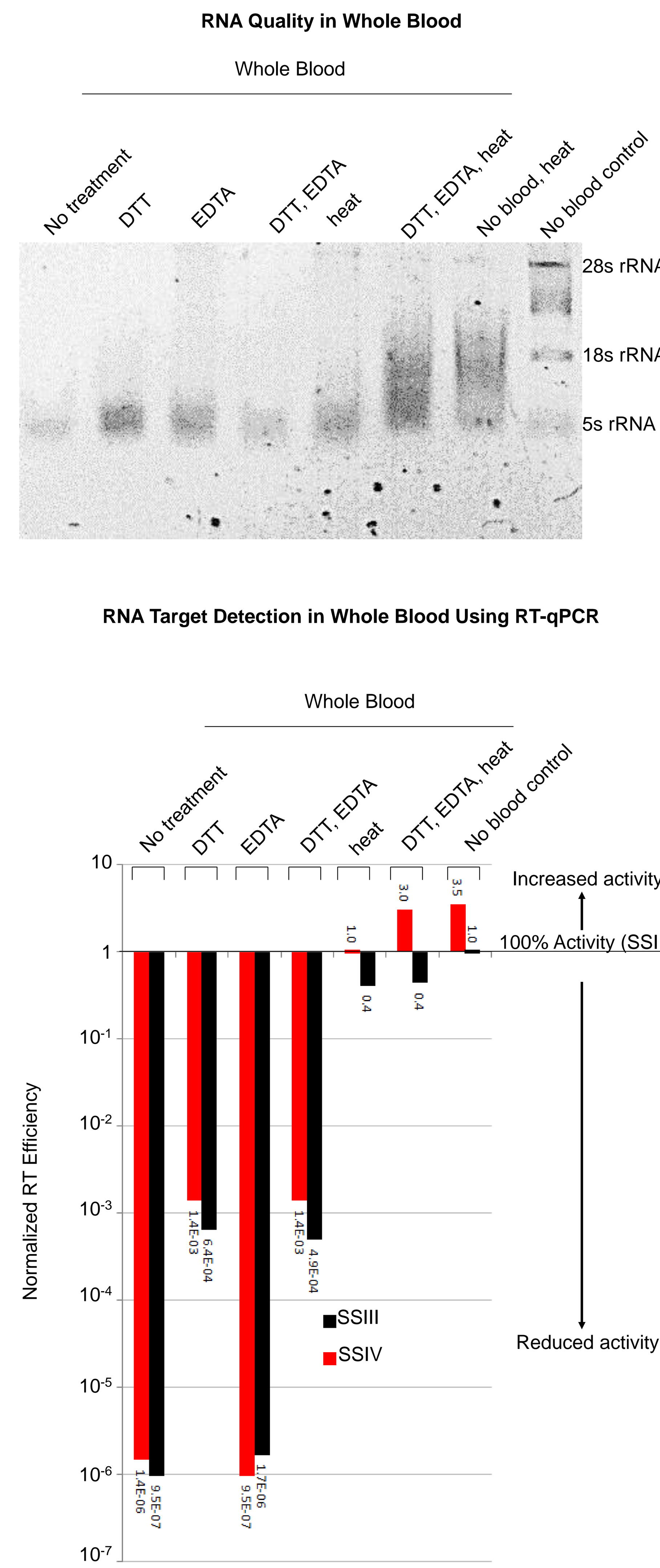
100 ng of total RNA from HeLa cells (Cat# AM7852) was used in a 20-µL SSIV reaction with oligo(dT)$_{20}$ according to provided protocol. Competitive products followed the manufacturer's recommended protocols. Inhibitors were added to total RNA prior to annealing to primer or addition of RT reaction mix. 10% of cDNA in the RT reaction composed 10% of the TaqMan® qPCR (Cat# 11785-01K) reaction volume which targeted the human TFRC (CD71) (transferrin receptor) gene (Cat# 4333770F). Ct values were normalized to SuperScript®IV using the equation: Normalized Y values=[2^(Ct$_{SSIV}$- Ct$_{SSIV}$)]/[2^(Ct$_{competitor}$- Ct$_{SSIV}$)]. SSIV consistently detects targets in HeLa RNA in the presence of inhibitors commonly found in sample preparation reagents and blood.

Figure 3. SuperScript® IV First-Strand cDNA Performance in the Presence of Inhibitors



500 ng 0.5-10 kb RNA ladder (Cat# 15623-200) was used in a 10-µL SSIV reaction with oligo(dT)$_{20}$ according to provided protocol. Competitive products followed the manufacturer's recommended protocols. Inhibitors were added to total RNA prior to annealing to primer or addition of RT reaction mix. First-strand cDNA were resolved by alkaline gel electrophoresis and cDNA was stained using SYBR-gold (Cat# S-11494). NaOH hydrolyzes all RNA resulting in only visualization of cDNA. SSIV consistently generates longer cDNA with higher yields in the presence of inhibitors found in FFPE (formalin), blood (hematin and heparin), and feces (bile salts).

Figure 4. RNA Detection in Whole Blood using SuperScript® IV



Human disease research may sometimes require the detection of RNA in whole blood. Whole blood contains a combination of inhibitors, including heparin, heme, and high concentrations of RNases. To investigate, the performance of SSIV in whole blood, 100 ng HeLa RNA was added to whole blood in reverse transcription reactions composed of 2.5% whole blood. Either untreated blood, addition of 5 mM DTT and/or 1 mM EDTA, and/or heat treatment was also tested to optimize RNA detection in blood. Heat treatment consisted of heating blood/RNA samples to 95C for 10 minutes. As a positive control, the same amount of HeLa RNA was used in a reverse transcription reaction in the absence of blood. Normalized Y values=[2^(Ct$_{SSIII\ no\ blood}$- Ct$_{SSIII\ no\ blood}$)]/[2^(Ct$_{competitor}$- Ct$_{SSIV}$)]. SSIV consistently detected more 18s rRNA target by TaqMan® qPCR in heat-treated whole blood than SSIII. The best results consisted of the addition of DTT, EDTA, heat treatment, and the use of SSIV, suggesting that the most inhibitory component in blood are RNases. Ct value with no blood is 14.

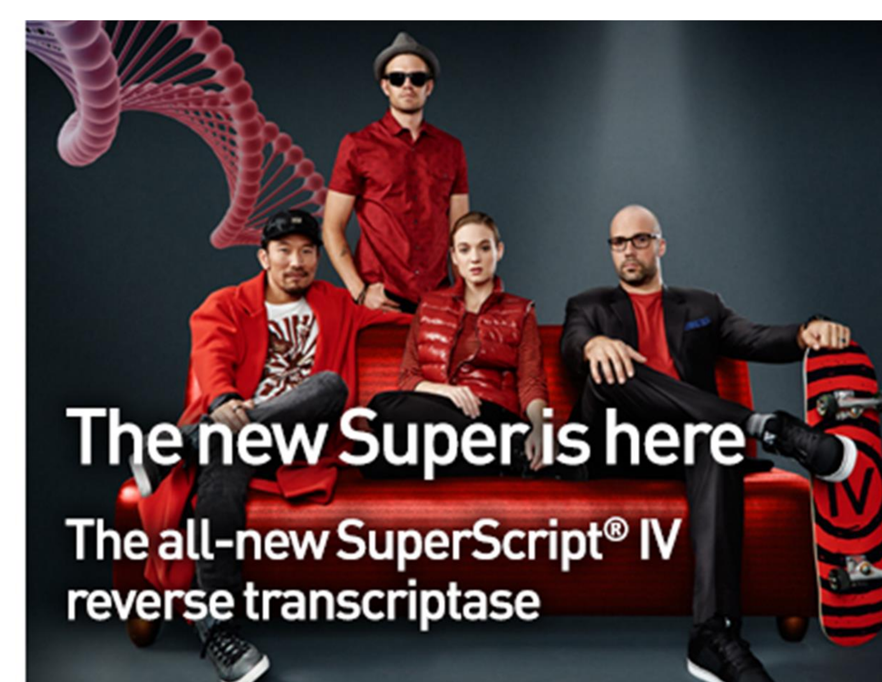
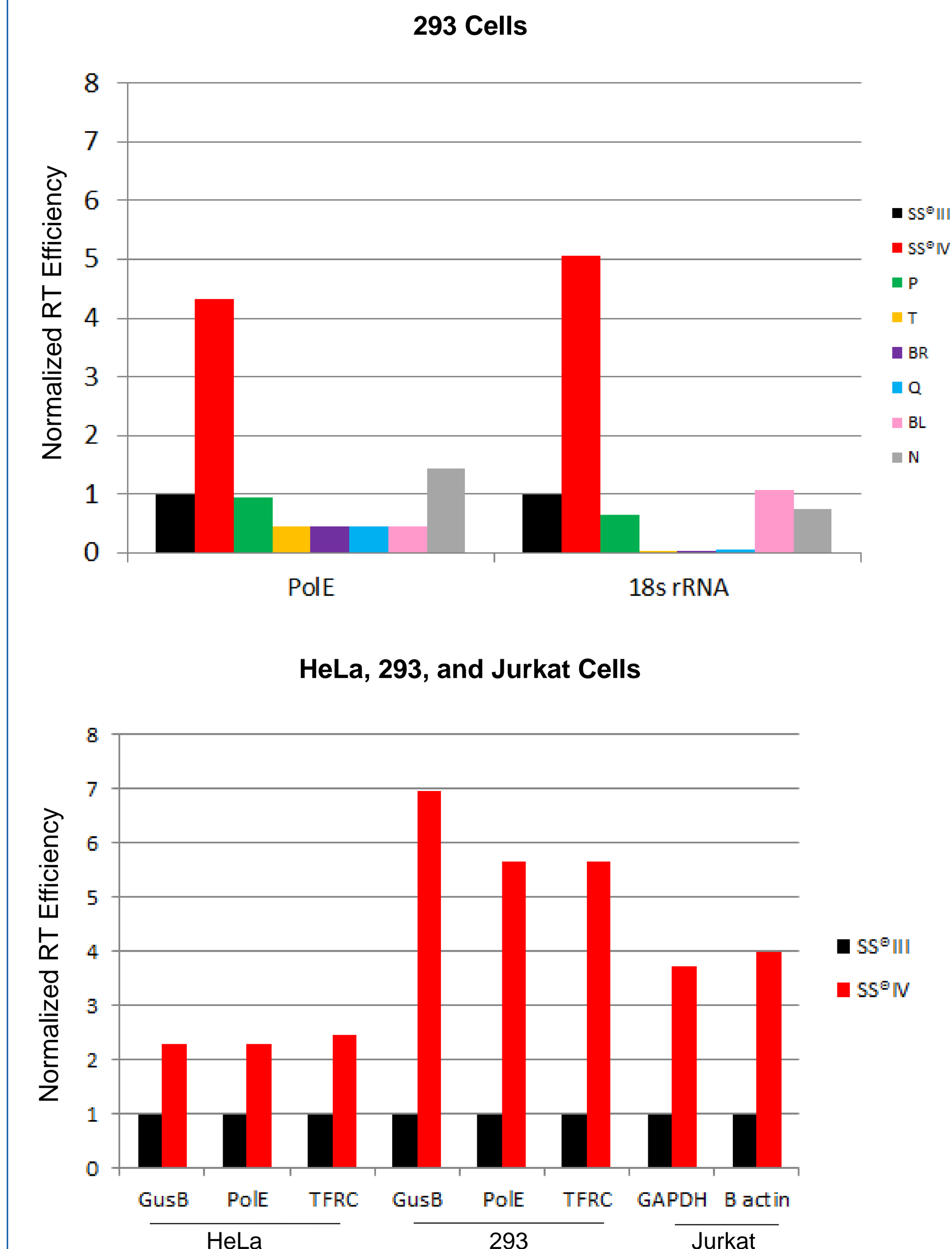


Figure 5. RNA Detection in Unpurified RNA



Pelleted cells were ground to a fine powder in liquid nitrogen. The powder was transferred to a microfuge tube and TE was added, vortexed, and centrifuged to pellet debris. Resulting clarified supernatant was transferred into a fresh microfuge tube. Prior to reverse transcription, EDTA and DTT were added to the supernatant to a final concentration of 1 mM and 5 mM respectively, and heated to 95 °C for 10 minutes. TaqMan® qPCR reactions were used to detect targets indicated in figures above. Ct values were normalized to SuperScript®IV using the equation: Normalized Y values=[2^(Ct$_{SSIV\ or\ SSIII}$- Ct$_{SSIV\ or\ SSIII}$)]/[2^(Ct$_{competitor}$- Ct$_{SSIV}$)]. SSIV consistently detects targets in unpurified RNA samples better than other commercial reverse transcriptases. Ct values for SSIV ranged between 25-35.

CONCLUSIONS

Thermo Fisher Scientific enables researchers to rapidly advance their studies by continuously evolving its reagents and tools for current and future research. The SuperScript®IV reverse transcriptase is an example of such a reagent. This enzyme is efficient in detecting RNA from samples containing inhibitors, such as alcohols, cell lysis reagents, salts, and those inherent in biological samples, such as those found in blood, feces, and FFPE RNA. Not only is this enzyme resistant to inhibitors, but it also possesses increased sensitivity such that RNA targets are detected from directly lysed human cell lines. Therefore, SuperScript®IV provides the most sensitive and reliable RNA analyses from the most challenging types of sample sources. Future studies will focus on more systematic and quantitative studies using unpurified RNA. Future considerations relating to SuperScript®IV reverse transcriptase will investigate more convenient formats for users running high throughput RT-qPCR and RT-PCR applications.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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