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

Authors: Blanca J Lam¹, Kevin Zhang¹, Joanna Guo¹, Lushen Li¹, Kimberly Wong Garcia¹, Asta Jokubkauskaite², Milda Kanusaitë³, Tomas Radzvilavičius², and Arunas Lagunavičius²

¹Thermo Fisher Scientific/Life Sciences Solutions, 5781 Van Allen Way, Carlsbad, California, USA, 92008
²Thermo Fisher Scientific/Life Sciences Solutions, V.A. Graiciuno 8, LT-02241 Vilnius, Lithuania

---

**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 increased difficulty in 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 alkali 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 gene. In the context of SuperScript® IV, the newest member of the SuperScript® family of reverse transcriptases, which is known for its robust performance and reliability in DNA synthesis. This new enzyme has been proven to function in experimentally challenging conditions that many scientific faces, such as in the presence of enzyme inhibitors found in reagents, cells, blood, feces, and FFPE samples. To demonstrate this enzyme's capabilities further, RNA transcripts were detected in unpurified RNA samples.

---

**REVERSE TRANSCRIPTION PROTOCOL**

Figure 1. Reverse transcriptase inhibitors

- **Inhibitors:** Alcohol (ethanol), chlorides, salts, guanidinium thiocyanate, ammonium acetate, cell lysis, detergents, solubilizers, heparin, hematin, bile salts, formalin.
- **Source:** Sample prep.

---

**RESULTS**

Figure 1. Reverse transcriptase inhibitors

- **SSV-05-10 mg/kg RNA ladder (Cat# 10532-200) was used in a 1:10 dilution SSV reaction with oligo(dT)12-4 according to provided protocol. Competitive products followed the manufacturer's recommended protocols. Inhibitors were added to total RNA prior to annealing to primer or to addition of reaction mix at room temperature. Samples were resolved by gel electrophoresis and λDNA was stained by SYBR® gold (Cat# S-1164). NTAq hydrolyzes all RNA resulting in very visualization of CNp. SSVIY consistently generates larger CNp with higher yields in the presence of inhibitors found in FFPE (formalin, blood and heparin), and so focus p53 allele.

---

**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 sample containing inhibitors, such as alcohol, cell lysates, reagents, and inhibitors 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 analysis from the most challenging samples of source samples. Future studies will focus on more systematic and quantitative studies using unpurified RNA. Future considerations relating to SuperScript®IV reverse transcriptase will provide more convenient formats for users running high throughput RT-qPCR and RT-PCR applications.

---

**ACKNOWLEDGEMENTS**

We would like to thank Toby Jordan and Samantha Li for their work and support in the development of SuperScript®IV.

---

**TRADEMARKS/LICENSING**

© 2015 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan® is a registered trademark of Roche Molecular Systems, Inc. under license. TTRiQ is a trademark of Molecular Research Center, Inc. Soluslyte® is a trademark of Genlantis, Inc.