Enhancing RT-PCR Efficiency: Reducing RT-Hold Times for Rapid Molecular Diagnostics

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Developing molecular Point-of-Care (mPOC) diagnostic assays is no simple task. While sensitive and specific, traditional RT-PCR protocols are not ideal for use in mPOC assays that demand significant decreases to turnaround time to keep patient time in the clinic or doctor's office as short as possible. For detection of RNA targets through RT-PCR, two distinct enzymatic steps have to be optimized. This note will focus on the first of these two steps, the reverse transcription, or RT, step. Optimization of the RT step to reliably convert target RNA into amplifiable DNA in a short time frame remains a significant challenge for assay developers.

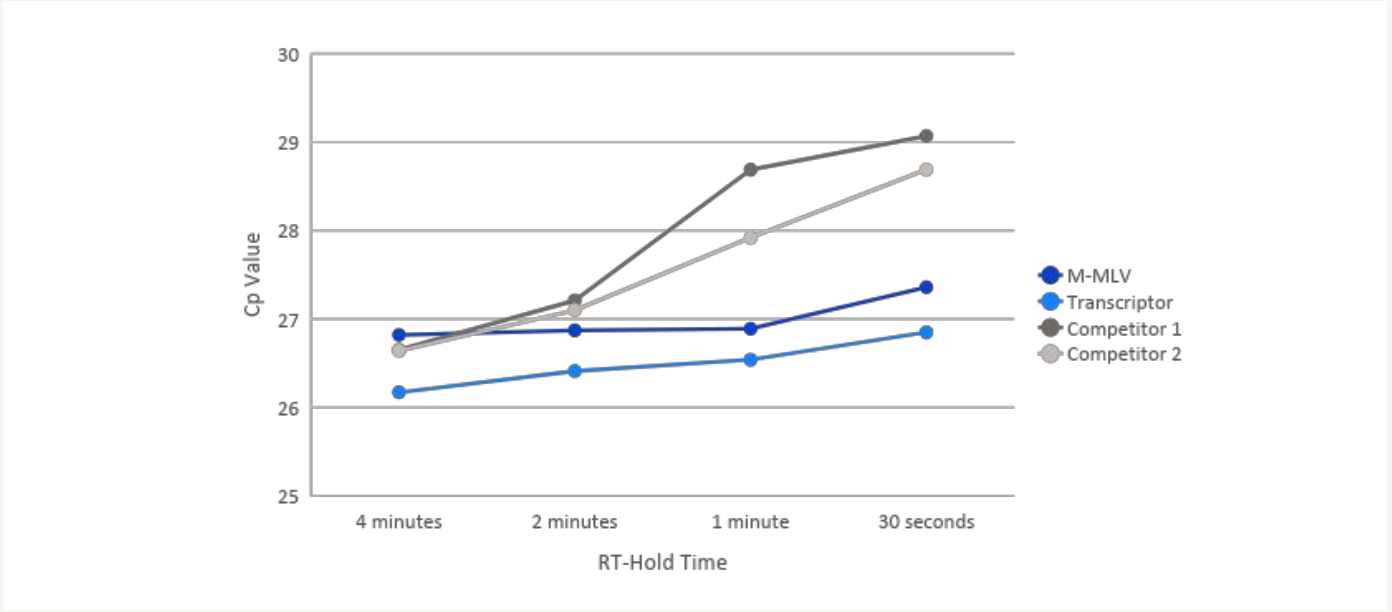
Traditional RT-PCR based diagnostics often require hold times in the range of 5 to 15 minutes, sometimes even longer. When considering POC testing where sites typically want answers in 15 to 30 minutes, these hold times are simply too long to meet that demand. To enable the use of shorter RT-hold times, reverse transcriptases need to generate enough cDNA to be detectable when RT-hold times well below five minutes are used. Throughout the rest of the note, we will demonstrate how the requirements for needing a short RT step can be met by using reverse transcriptase enzymes from Roche CustomBiotech.

CustomBiotech RT enzymes excel when using short RT-hold times

The need for long RT-hold times remains a key barrier to the use of RT-PCR in rapid mPOC assays, including in 1-step protocols. Given that one of the primary goals of most point-of-care (POC) assay designers is to reduce overall turnaround times to "as short as possible," every minute is crucial. Naturally, this has led to efforts to reduce the RT-hold time without sacrificing assay performance. Using an RT enzyme with a demonstrated ability to work under short RT-hold conditions represents one method of reducing the RT-hold time required. Roche CustomBiotech offers wild-type enzymes, such as M-MLV and Transcriptor, as well as high performance mutant enzymes, all of which are capable of meeting the demand for short hold times.

Figure 1 shows the difference in Cp values obtained when using different RT-hold times. At the 4-minute mark, all four enzymes generated roughly the same amount of cDNA, as evidenced by a tight grouping of the Cp values. As shorter RT-hold times were tested, the Roche M-MLV and Transcriptor RTs were able to keep a high level of cDNA synthesis while the competitor enzymes showed a more significant reduction in cDNA synthesis as evidenced by lower Cp values. When the RT-hold time was reduced from 4 minutes to 30 seconds, both Roche enzymes showed between a 1.1 and 1.2 shift in Cp value, indicating an approximate reduction of cDNA generation by only 2-2.5x. Over the same two points, the competitor enzymes showed larger Cp value shifts between 2.5 and 3, which leads to a reduction in cDNA generation of 5.5-8x.

Figure 1:

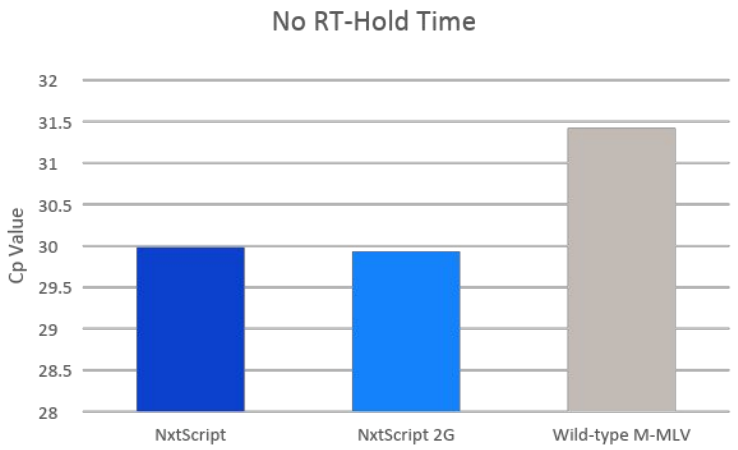


Comparison of Cp values using various RT-hold times for Roche M-MLV and Transcriptor RTs compared to two competitor RT enzymes

CustomBiotech M-MLV mutants work without an RT-hold step

Reduction of RT-hold times to as low as 30 seconds is a significant step forward in reducing mPOC turnaround times. Even so, every second counts in the field. This led us to test whether Roche enzymes were capable of converting target RNA into amplifiable DNA in the absence of any traditional hold step. Interestingly, NxtScript RT, NxtScript 2G RT, and a competitor wild-type M-MLV were all capable of producing detectable amounts of cDNA under the challenging condition of zero hold time. However, as can be seen in Figure 2, our next generation M-MLV-based mutant enzymes, NxtScript and NxtScript 2G, excelled in comparison to the wild-type M-MLV. These enzymes showed a clear 1-1.5 Cp advantage over the wild-type enzyme indicating an at least 2-fold greater amount of cDNA generation.

Figure 2:



Cp Values obtained when omitting the RT-hold step for NxtScript, NxtScript 2G, and the competitor wild-type M-MLV

Conclusion

Reducing turnaround time for mPOC assays is a difficult task. The extra RT step involved in RT-PCR based assays, when compared to PCR based assays, significantly increases the magnitude of this challenge. To help navigate these obstacles, Roche CustomBiotech offers a wide selection of reverse transcriptase enzymes, including: M-MLV RT, Transcriptor RT, NxtScript RT, and NxtScript 2G RT, that are capable of running fast to reduce overall turnaround times.

Ordering Information

Product	Pack Size	Catalog Number
M-MLV RT GMP Grade	200 kU	04707486103
Transcriptor Reverse Transcriptase	Custom Fill	03531252103
NxtScript Reverse Transcriptase	Custom Fill	07051166103
NxtScript 2G Reverse Transcriptase	Custom Fill	09085220103
Related products		
KAPA3G HotStart Master Mix	Custom Fill	09084711103
AptaTaq Genotyping Master	Custom Fill	05890152103

SCAN ME

Interested in learning about other ways our reverse transcriptases or other CustomBiotech enzymes can reduce turnaround time or simplify your assay design process?

Follow the QR code below to find more information on our website.

Regulatory Disclaimer
For further processing only.

AptaTaq Genotyping Master:
For further processing into IVD products and medical devices only.

Transcriptor Reverse Transcriptase:
For customers in the European Economic Area: Contains SVHC: octyl/nonylphenol ethoxylates. For further processing on its own or in a mixture as part of an IVD method and under controlled conditions only acc. to Art. 56 (3) and 3 no. 23 REACH Regulation.

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