

Simplifying Storage for mPOC Assays: Lyophilization and Room Temperature Storage of Mutant M-MLV Enzymes

November 2024

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Developing molecular Point-of-Care (mPOC) diagnostic assays is no simple task. While sensitive and specific, traditional RT-PCR protocols and techniques are not ideal for use in mPOC assays that demand significant changes in the way reagents are handled and stored. This note will focus on the first of the two enzymatic steps in an RT-PCR assay, the reverse transcription, or RT, step. Typically, reverse transcriptases have to be stored at frozen

temperatures to prevent loss of activity over time. This requirement forces mPOC testing sites to keep and maintain freezers they otherwise would not need which take up space and consume resources that could be better used elsewhere. Overcoming this barrier necessitates the use of enzymes that can be stored at temperatures above freezing. Below, we will demonstrate how NxtScript and NxtScript 2G can help simplify storage conditions for mPOC applications.



Room temperature stability of NxtScript and NxtScript 2G

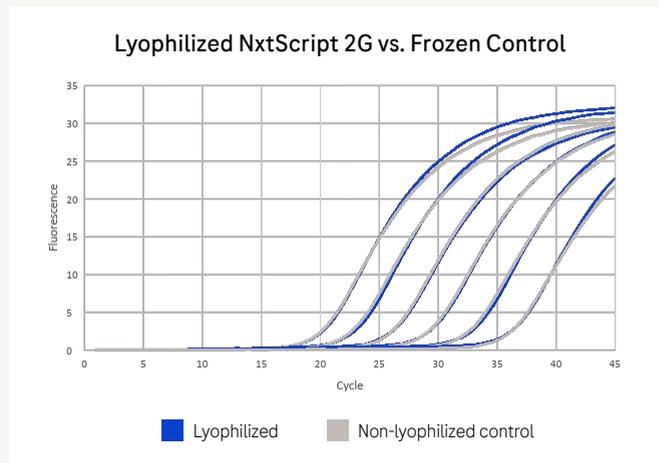
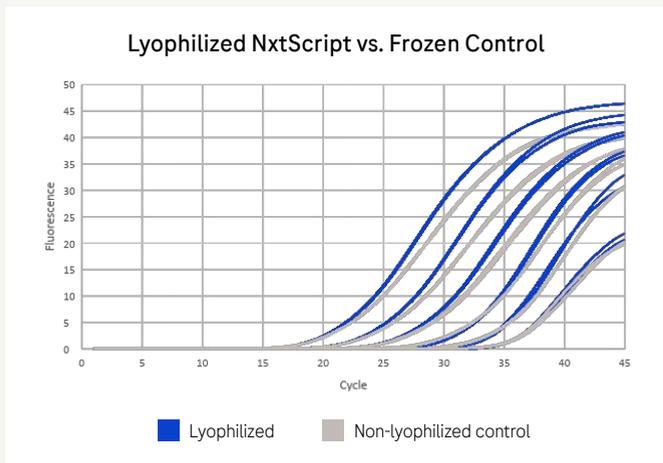
Reverse transcriptases, DNA polymerases, and master mixes usually require storage at $-20\text{ }^{\circ}\text{C}$ for long-term stability. This cold chain requirement can create a significant burden for storage of assay material in mPOC settings where freezers are often not readily available or are cumbersome to maintain. In some cases, storage under refrigerated ($4\text{-}8\text{ }^{\circ}\text{C}$) conditions may even be a challenge. One way of mitigating cold chain challenges is by drying the material into a room temperature stable solid, typically through

lyophilization. Regardless of the drying method, some components commonly found in RT-PCR reagents make drying difficult, sometimes even impossible, without modification to the buffer conditions. Glycerol is a common storage buffer component used to improve enzyme stability, however its presence can be detrimental to the lyophilization process. For reagents to be dry-down compatible, two strategies are employed. Either the enzyme must be in such high concentration that the glycerol content is effectively

zero in the final reaction mix, or the enzyme is formulated in a glycerol free format. The formulation of the NxtScript and NxtScript 2G reverse transcriptases utilized these two approaches, respectively. Figure 2 below demonstrates that both NxtScript RT (Figure 1A) and NxtScript 2G RT (Figure 1B) retain activity following lyophilization. Both enzymes almost perfectly match control reactions

performed using material that had never been dried. Optimized lyophilization protocols generally confer a great deal of stability, and, as evidenced by longer-term studies on other enzymes in the Roche CustomBiotech RT-PCR portfolio, can possibly allow for retention of enzyme activity after long-term storage at room temperature.^{1,2}

Figure 1:



A) 1-Step RT-PCR Amplification curves when using Lyophilized NxtScript reconstituted in PCR Master Mix (Blue) and compared to control reactions using non-lyophilized NxtScript (Gray).

B) 1-Step RTPCR Amplification curves when using Lyophilized NxtScript 2G reconstituted in PCR Master Mix (Blue) and compared to control reactions using non-lyophilized NxtScript 2G (Gray).

1. [KAPA3G HotStart DNA Polymerase – made for IVD applications](#)
2. [Lyophilized reagents for molecular point-of-care diagnostics](#)

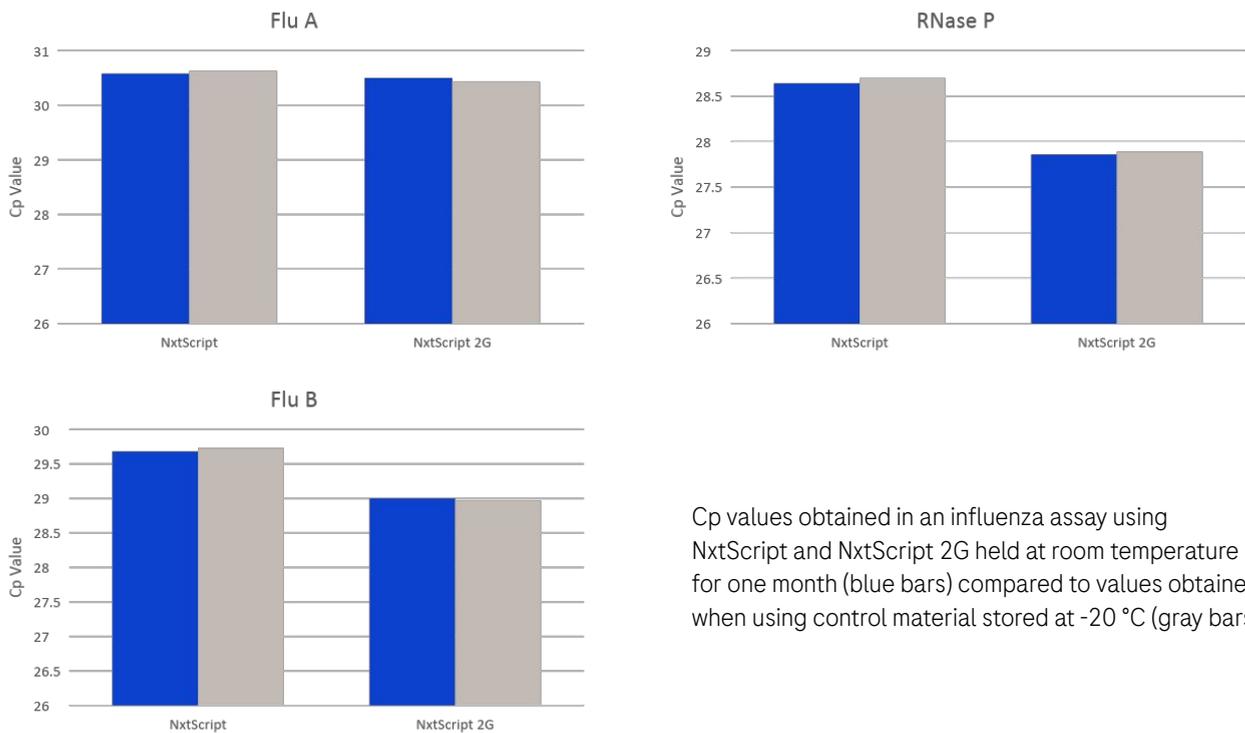


Liquid stability of M-MLV Mutants

While lyophilization is a powerful tool to create room temperature stable mixes and reagents, it requires specialized equipment, is difficult to optimize, and is often expensive. Optimally, reagents would be stable at room temperature in a liquid format, yet long-term room temperature stability of liquid reaction mix, or even just enzymes alone is likely many years away. A cumbersome drying process could potentially be avoided by relying on reagents with short or medium term room temperature or refrigerated stability. For example, it could be possible to hold materials under

frozen conditions during warehousing, but after distribution to the end users allow for refrigerated or room temperature storage for a shorter period of time. NxtScript RT and NxtScript 2G RT could potentially work in this type of process. Figure 2 shows the Cp values obtained for three different targets in a triplex influenza assay after the enzymes were stored in a lab drawer at room temperature for one month compared to values obtained when using material that was stored at -20 C.

Figure 2:



Cp values obtained in an influenza assay using NxtScript and NxtScript 2G held at room temperature for one month (blue bars) compared to values obtained when using control material stored at -20 °C (gray bars)



Conclusion

Solving the problem of material storage for mPOC assays is not a simple task. The extra RT step involved in RT-PCR based assays, when compared to PCR based assays, significantly increases the difficulty in managing cold chain storage conditions. The introduction of RT enzyme into the mPOC workflow means that two separate enzymes, the DNA polymerase and reverse transcriptase, have to be stabilized to enable storage at temperatures above freezing. To help navigate the storage obstacle, Roche CustomBiotech offers M-MLV mutant enzymes, NxtScript and NxtScript 2G that are compatible with dry down techniques, and even offer limited room temperature stability in liquid form, retaining activity even when stored under less than ideal conditions for up to a month.

Ordering Information

Product	Pack Size	Catalog Number
M-MLV RT GMP Grade	200 kU	04707486103
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



Interested in learning about other ways KAPA3G HotStart DNA Polymerase or other Roche CustomBiotech enzymes can reduce the turnaround time of your assay?

Follow the QR code to find more information on our website. Additional data on the inhibitor tolerance, speed, robustness, and other performance parameters of KAPA3G HotStart DNA Polymerase are available upon request.

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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