Lyophilization & Post-Lyophilization User Guideline

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The guidelines in this document can help users avoid problems in lyophilization. For storage and stability, expiry and general handling of these product pre-lyophilization, please refer to the individual Product Handling Guides.

Safety precautions:
Read and understand the SDS (Safety Data Sheets) before handling the reagents. Copies of these SDSs are available on our website or upon request.

There are several advantages for lyophilization, including room temperature shipping and storage, extended shelf-life and increased flexibility in sample volume. In order to be compatible with lyophilization however, enzyme preparations must be glycerol-free and include specialized lyophilization-excipients that preserve the mixture as it is exposed to various lyophilization conditions including freezing, temperature ramps, vacuum and dehydration. An ideal lyophilization formulation should stabilize an enzyme in a freeze-dried format and allow very fast rehydration and reactivation of the enzyme preparations, without impacting its performance post rehydration. The MDX product listed in table 1 are suitable for lyophilization.

Table 1. Lyophilization compatible products

Product	Catalog number
Glycerol-Free Taq HS 50 U/µL	MDX011
Aptamer Taq HS (Glycerol-Free)	MDX015
Glycerol-Free Bst	MDX017
Lyo-Ready qPCR Mix	MDX021
Lyo-Ready qPCR Buffer, 2.5x	MDX022
Lyo-Ready qPCR Mix 2.6x	MDX023
Lyo-Ready 1-Step RT-qPCR Mix	MDX024
Enzyme Dilution Buffer, 10x	MDX011, MDX024, MDX042 and MDX062
Lyo-compatible MMLV-RT*	MDX042
Lyo-Ready 1-Step RT-qPCR Buffer	MDX052
Lyo-Ready qPCR Buffer w/o Excipients, 4x	MDX061
Lyo-Ready 1-Step RT-qPCR Virus Mix	MDX062

*In 50% Glycerol (<0.01% in final reaction)

Critical Temperatures

Glass transition for frozen state (Tg') Collapsing (Tc) and Glass Transition for dried state (Tg^d) critical temperatures for Lyo-Ready qPCR Mix, Lyo-Ready 1-Step RT-qPCR Mix and Lyo-Ready 1-Step RT-qPCR Virus Mix are listed in table 2.

Table 2. Tg', Tc and Tg^d critical temperatures

Types of Mix	Tg'	Tc	Tg^d
Lyo-Ready qPCR Mix	-36.3°C	-33.6 °C	45 °C
Lyo-Ready 1-Step RT-qPCR Mix	-33 °C	-29.4 °C	45 °C
Lyo-Ready 1-Step RT-qPCR Virus Mix	-33 °C	-29.4 °C	45 °C

Lyophilization

- The lyophilization cycle protocol in Table 2 is suitable for lyophilization of the Lyo-Ready qPCR mix and 1-step RT-qPCR mix in standard PCR tubes and plates. These parameters are provided as a guidance only and should be optimized to different user formats and systems.
- An annealing step can be added during the freezing step to assist crystallization of amorphous material.
- Combined primary and secondary drying time can be extended up to 24 hours.
- For product containing excipients, there should be no need to add any further excipients to assist lyophilization.

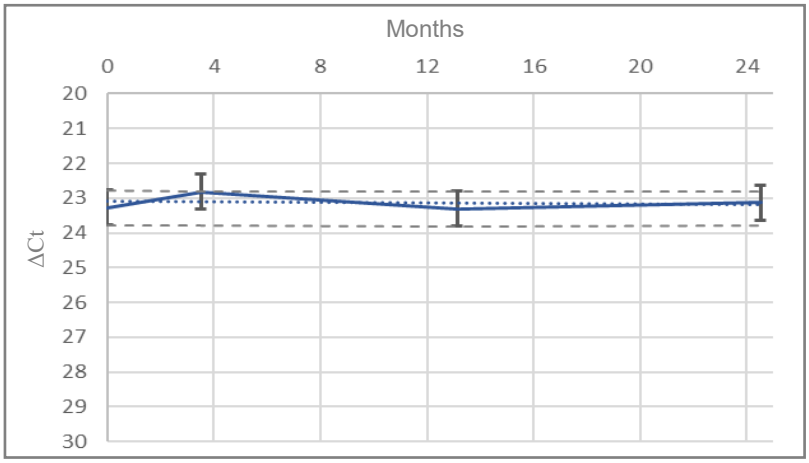
Table 3. Lyophilization guidelines

Step	Temperature	Time	Description
Freezing	+4 °C	10 min	Hold
	-45 °C	1.0 °C/min	Ramp
Primary Drying	-45 °C	180 min	Hold
	-40 °C	0.5 °C/min	Ramp
	-40 °C	720 min	Hold
Secondary Drying	+25 °C	0.5 °C/min	Ramp
	+25 °C	240 min	Hold

Post-Lyophilization

- Lyophilized qPCR and 1-step RT-qPCR mixes must be handled in a humidity-controlled environment of <5% humidity to ensure storage stability.
- For maximum shelf-life, we suggest packaging lyophilized material under inert gas conditions (e.g. nitrogen or argon) and insert a desiccant sachet to improve stability.
- Pouches should be heat-sealed and labelled.

Graph 1. Stability of the lyophilized Lyo-Ready Mixes at ambient temperature



Results illustrate that the Lyo-Ready qPCR Mix is active for up to 24 months at room temperature. Lyo-Ready 1-Step RT-qPCR Mix and Lyo-Ready 1-Step RT-qPCR Virus Mix have a projected stable activity up to 12 months. Method error is shown as timepoint standard deviation. Permissible activity interval is shown as dashed lines.

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Technical Support

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