

# Lyophilization & Post-Lyophilization User Guideline



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.

## Critical Temperatures

Glass transition for frozen state ( $T_g'$ ) Collapsing ( $T_c$ ) and Glass Transition for dried state ( $T_g^d$ ) critical temperatures for Lyo-Ready™ Direct RNA/DNA LAMP Stool is listed in table 1.

Table 1.  $T_g'$ ,  $T_c$  and  $T_g^d$  critical temperatures

Types of Mix	$T_g'$	$T_c$	$T_g^d$
Lyo-Ready™ Direct RNA/DNA LAMP Stool	-33 °C	-29.4 °C	45 °C

## Lyophilization

- The lyophilization cycle protocol in Table 1 is suitable for lyophilization of the Lyo-Ready™ Direct RNA/DNA LAMP Stool 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.

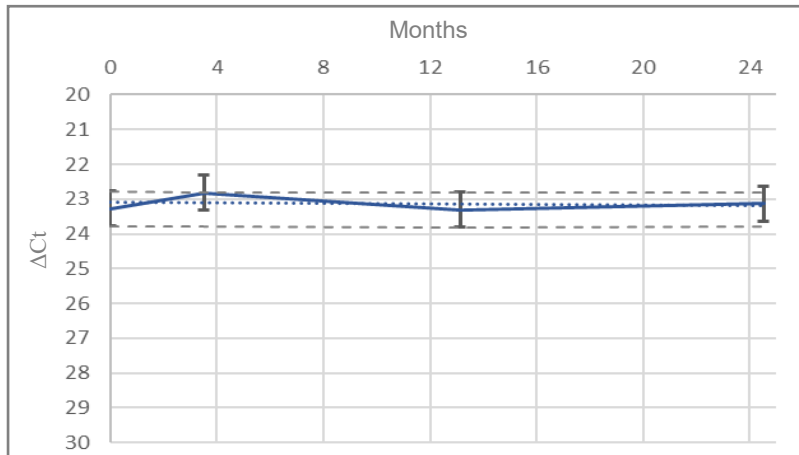
Table 2. Lyophilization guidelines

Step	Temperature	Time	Description
Freezing	+4 °C	10 min	Hold
	-45 °C	1.0 °C/min	Ramp
	-45 °C	1.0 °C 120 min	Hold
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 LAMP and RT-LAMP 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™ LAMP Mix is active for up to 24 months at room temperature. Lyo-Ready™ Direct RNA/DNA LAMP Stool has a projected stable activity up to 12 months. Method error is shown as timepoint standard deviation. Permissible activity interval is shown as dashed lines.