

Lyophilization & Post-Lyophilization User Guideline: Lyo-Cakes and Lyo-Beads



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.

Lyo-Cakes Lyophilization

- The lyophilization cycle protocol in Table 1 is suitable for lyophilization of the Lyo-Ready mixes 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 1. Lyo-Cakes 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

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 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. T_g' , T_c and T_g^d critical temperatures

Types of Mix	T_g'	T_c	T_g^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

Post-Lyophilization

- Lyophilized 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.

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Lyo-Beads Lyophilization

- The lyophilization cycle protocols in Table 3 is suitable for lyophilization of Lyo-Ready mixes in lyophilized bead format. These parameters are provided as a guidance only and should be optimized to different user formats and systems.
- The product already contains excipients, therefore, there should be no need to add any further excipients to assist lyophilization.

Table 3. Lyo-Beads lyophilization guidelines

Step	Temperature	Time	Description	Vacuum Set Points
Shelf Pre-Freezing	-40 °C	n/a	Pre-Freezing	
Freezing	-45 °C	0.5 °C/min	Ramp	
	-45 °C	10 min	Hold	
Evacuation	-55 °C (Condenser)			75 mTorr (100 µbar)
Primary Drying*	-45 °C	180 min	Hold	37.5 mTorr (50 µbar)
	-40 °C	0.5 °C/min	Ramp	
	-40 °C	900 min	Hold	
Secondary Drying	+22 °C	0.5 °C/min	Ramp	37.5 mTorr (50 µbar)
+22 °C	240 min			Hold

*Primary drying time could be extended to up to 24h depending on quantity of sample that is lyophilized

Post-Lyophilization

- Lyophilized 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.