LAMP

Assessing Lyo-ready Bst DNA Polymerase stability after air-drying and lyophilization

Introduction

Molecular diagnostic assay developers use lyophilization to maximize a product's shelf life and stability at room temperature. Most raw materials for assay development are supplied in a conventional liquid formulation where enzymes usually contain up to 50% glycerol, which serves as a cryoprotectant and stabilizes enzymes. Therefore, most conventional enzyme formulations are incompatible with drying. The Invitrogen™ Lyo-ready Bst DNA Polymerase meets the high-performance requirements of loop-mediated isothermal amplification (LAMP)-based assays and is also compatible with drying. The Lyo-ready Bst DNA Polymerase allows for the development of fast and sensitive LAMP assays in dry formats.

The most widely used technique for drying proteins and biological materials is freeze-drying, or lyophilization. While this technique is the gold standard for drying different biological materials, lyophilization has shortcomings, including the need for expensive equipment, long drying time, etc. Recently, air-drying has started to gain popularity. This method is based on using a simple heating oven to evaporate water. This eliminates the need for expensive equipment and the process is also much faster. However, there is concern regarding the stability of enzymes after prolonged exposure to a hot environment.

Here we evaluated the stability of Lyo-ready Bst DNA Polymerase in either lyophilized or air-dried format and the ability of each to detect RNA from SARS-CoV-2. The protocol demonstrates that neither air-drying nor lyophilization impairs the stability of Lyo-ready Bst DNA Polymerase, and that RNA from SARS-CoV-2 can be detected via a reverse transcription (RT)-LAMP reaction at the same level of sensitivity prior to drying by either method.

Materials and methods

Enzyme preparation

- Air-drying
  - Lyo-ready Bst DNA Polymerase was divided in 10 µL aliquots into Eppendorf™ 1.5 mL tubes and air-dried for 2.5 hr at 50°C in an oven (Binder BD53).
  - The dried enzyme samples were placed into plastic bags with desiccant and stored at 25°C for 13 weeks.
  - Each dried enzyme sample was reconstituted with nuclease-free water to a final volume of 10 µL.
- Lyophilization
  - Lyo-ready Bst DNA Polymerase was divided in 10 µL aliquots into an Applied Biosystems™ MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL and lyophilized using the SP VirTis™ Advantage Pro™ XL Freeze Dryer (SP Industries) following the parameters in Table 1.
  - The dried enzyme samples were placed into plastic bags with desiccant and stored at 25°C for 13 weeks.
  - Each dried enzyme sample was reconstituted with nuclease-free water to a final volume of 10 µL.

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Polymerase activity

- Polymerase activity was measured by incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction for 30 min at 60°C.

RT-LAMP functional test

- The RT-LAMP reaction mix for the detection of SARS-CoV-2 RNA was prepared on ice in MicroAmp Fast Optical 96-Well Reaction Plates. The final volume of a single RT-LAMP reaction was 25 µL.
- Sterile-filtered pipette tips were used to prevent aerosol contamination. Table 2 contains the reaction setup for SARS-CoV-2 RNA detection, including the materials, reagents, and buffers used, and their respective volumes, final concentrations, and supplier information.
- All RT-LAMP reactions for viral RNA detection were performed using an Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System.
- One-step cycling conditions were applied to all RT-LAMP reactions following the parameters in Table 3.
- Reaction kinetics and specificity of amplified products were analyzed using Applied Biosystems™ QuantStudio™ Real-Time PCR Software for the QuantStudio 7 Flex system.
- Specificity of the RT-LAMP was determined by melting curve analysis.

Table 1. Parameter setup for lyophilization.

Step	Temperature (°C)	Pressure (mTorr)	Time (min)
Freezing	–50	–	60
Drying	–50	700	480
	–20	150	480
	10	150	60
Storage	4	150	–

Table 3. One-step cycling protocol for RT-LAMP.

Step	Number of cycles	Temperature	Time
Amplification	50	65°C	30 sec
Inactivation	1	95°C	2 min
Melt curve	–	60–95°C	–

Table 2. Reaction setup for SARS-CoV-2 RNA detection.

Material	Volume (µL) for 25 µL reaction	Final concentration	Supplier	Cat. No.
Lyo-ready Bst DNA Polymerase, 40 U/µL	0.15	0.24 U/µL	Thermo Fisher Scientific	A56657
Lyo-ready SuperScript IV Reverse Transcriptase, 200 U/µL	0.13	1 U/µL	Thermo Fisher Scientific	EP164BSMP*
Lyo-ready RNaseOUT RNase Inhibitor, 40 U/µL	0.4	1.6 U/µL	Thermo Fisher Scientific	EO2521SMP*
dNTP mix, 25 mM each	1.4	1.4 mM	Thermo Fisher Scientific	R1121
Betaine, 5 M Solution	5	1 M	Thermo Fisher Scientific	Supplied with A56657
200 mM $MgCl_2$	0.75	6 mM	Thermo Fisher Scientific	Supplied with A56657
Reaction Buffer, 10X	2.5	1X	Thermo Fisher Scientific	Supplied with A56657
Primer FIP1 [1]	1.1	1.6 µM	Metabion	–
Primer BIP1 [1]		1.6 µM	Metabion	–
Primer F1 [1]		0.2 µM	Metabion	–
Primer B1 [1]		0.2 µM	Metabion	–
Primer LoopF1 [1]		0.4 µM	Metabion	–
Primer LoopB1 [1]		0.4 µM	Metabion	–
5 mM SYTO 9 Green Fluorescent Nucleic Acid Stain diluted in nuclease-free water to 50 µM	2.5	5 µM	Thermo Fisher Scientific	S34854
Twist Synthetic SARS-CoV-2 RNA Control	2	1,000 copies/reaction	Twist Bioscience	–
Water, nuclease-free	9.08	–	Thermo Fisher Scientific	R0581

* Lyo-ready products are available through custom commercial supply. For more information, please visit thermofisher.com/lyo-ready

Results and discussion

To assess the stability of Lyo-ready Bst DNA Polymerase, air-dried and lyophilized samples were incubated for 13 weeks at 25°C, and polymerase activity was measured at different time points. It is typical for a dried (both air-dried and lyophilized) enzyme to lose 20–30% activity compared to its nondried form. As shown in Figure 1, both air-dried and lyophilized samples remained stable with minimal change in polymerase activity during storage. The ratios of polymerase activity for dried and nondried samples were calculated, indicating no substantial loss of enzyme activity after drying and storage, outside of the typical expected loss from drying.

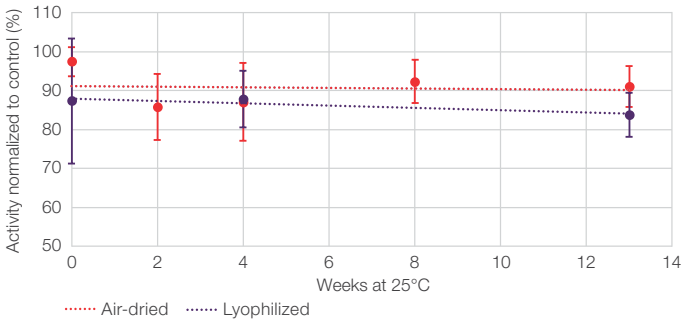

Figure 1. Stability of polymerase activity of air-dried and lyophilized Lyo-ready Bst DNA Polymerase after prolonged storage at 25°C. Air-dried and lyophilized Lyo-ready Bst DNA Polymerase samples were incubated at 25°C for 13 weeks. Polymerase activity was measured at different time points: 0, 2, 4, 8, and 13 weeks for the air-dried formulation (red), and 0, 4, and 13 weeks for the lyophilized enzyme (purple). The polymerase activity of dried samples was compared to the polymerase activity of the control enzyme prior to drying, which was stored at –20°C, and the ratios of activity were calculated. Three measurements were repeated, and the standard deviation was calculated. The horizontal trendlines (dotted line) indicate minimal stability change during storage.

To further investigate the functional performance of the enzyme, RT-LAMP reactions were performed using air-dried and lyophilized Lyo-ready Bst DNA Polymerase samples after 13 weeks of incubation at 25°C. The results, as shown in Figure 2, demonstrate that both air-dried and lyophilized Lyo-ready Bst DNA Polymerase samples successfully detected SARS-CoV-2 RNA after 13 weeks of storage at 25°C. The amplification signal appeared after ~5 minutes of amplification, and the negative control signal appeared no earlier than 14 minutes, confirming that the drying methods did not affect sensitivity or specificity.

Conclusion

Lyo-ready Bst DNA Polymerase exhibits stability after air-drying and lyophilization without additional additives. The enzyme activity remained stable and was within measurement variation compared to the nondried formulation, while the RT-LAMP reaction retained speed and sensitivity for SARS-CoV-2 detection. Lyo-ready Bst DNA Polymerase's compatibility with different drying methods makes it an ideal choice for point-of-care and field-based applications, providing reliable and cost-effective solutions for SARS-CoV-2 detection and other molecular diagnostic assay development.

Reference

1. Park G-S, Ku K, Baek S-H, et al. (2022) Development of Reverse Transcription Loop-Mediated Isothermal Amplification Assays Targeting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *J Mol Diagn* 22(6):729-735. doi:10.1016/j.jmoldx.2020.03.006

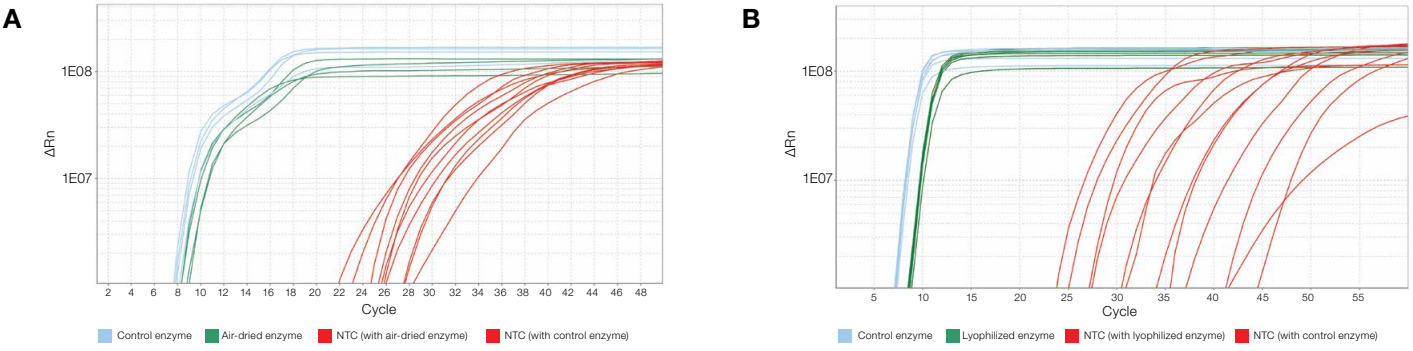

Figure 2. Detection of SARS-CoV-2 RNA using air-dried and lyophilized Lyo-ready Bst DNA Polymerase. RT-LAMP was performed with 1,000 copies of SARS-CoV-2 RNA using **(A)** air-dried and **(B)** lyophilized enzymes. Amplification was compared to the enzyme before drying (control, blue); the nontemplate controls (NTC) are depicted in red and the air-dried **(A)** and lyophilized **(B)** enzymes are depicted in green. The results demonstrate that there was no loss in speed, sensitivity, or specificity after drying with either method and long-term storage at 25°C.

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