Stabilization of RT-qPCR Reagents at Ambient Temperatures by PCRstable™ Technology

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Introduction

The need for cold storage of PCR-based test reagents makes the use of these reagents difficult in regions where access to freezers is either non-existent or undependable. PCRstable, a novel PCR reagent stabilization technology, is a reliable and cost effective alternative to cold storage. It was developed to stabilize PCR, qPCR and RT-qPCR reagents in dry or liquid format at ambient temperatures. In this study, we use PCRstable technology to combine the RT-qPCR reagents with proprietary biostability compounds in a simple air dried format. Following accelerated aging procedures at elevated temperatures, we performed RT-qPCR assays to assess the abilities of the stabilized reagents to perform their desired reactions. We demonstrate that the PCRstable technology successfully stabilizes all RT-qPCR reagents at ambient temperatures.

Materials and Methods

The specified RT-qPCR reagents were combined with proprietary stabilizers, incubated in a dry format at elevated temperatures, and tested at defined time points. The dried reaction mixtures were rehydrated with the appropriate components to complete the specified RT-qPCR assays. Depending on the assay, either gel electrophoresis or Ct and melt curve analysis (data not shown) were analyzed to assess the ability of the stabilized reagents to perform their desired functions. Each condition was run in triplicate and compared to a frozen positive control.

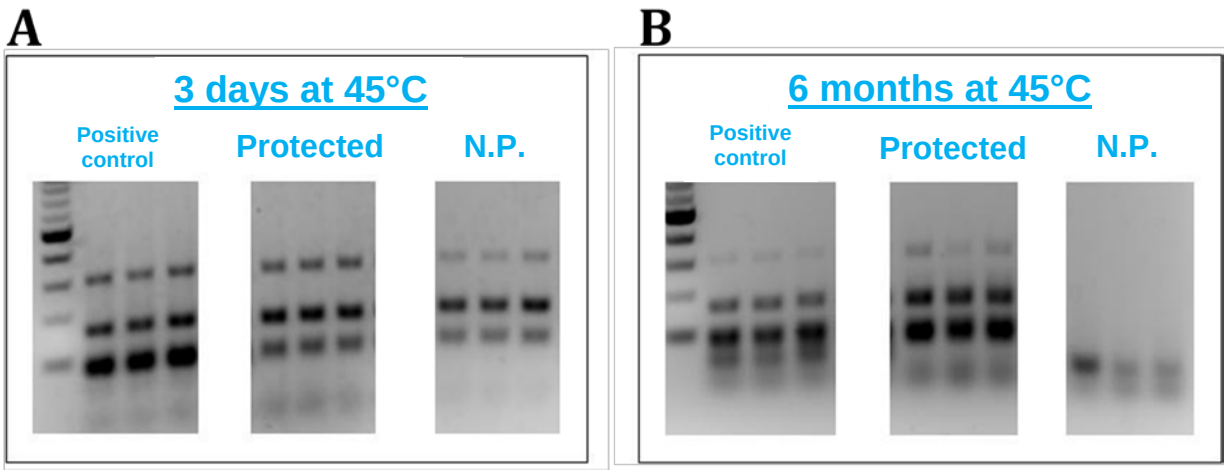

Figure 1. Multiplex RT-PCR assays with reverse transcriptase, primers, and dNTPs stabilized in a dried format by PCRstable technology. Reverse transcriptase, three sets of primers and dNTPs were dried in the presence (Protected) or absence (N.P.) of PCRstable formulations, and were stored at 45°C for accelerated aging studies. After the specified storage times, the reagents were rehydrated and their stabilities were assessed by reverse transcription followed by multiplex PCR amplification of 3 human TATA binding protein amplicons using a human RNA as a template. Reagents stored at -20°C were used as positive controls. Results show that PCRstable formulations stabilize the above reagents in a dried format for > 2 years at ambient temperatures based on the accelerated aging studies.

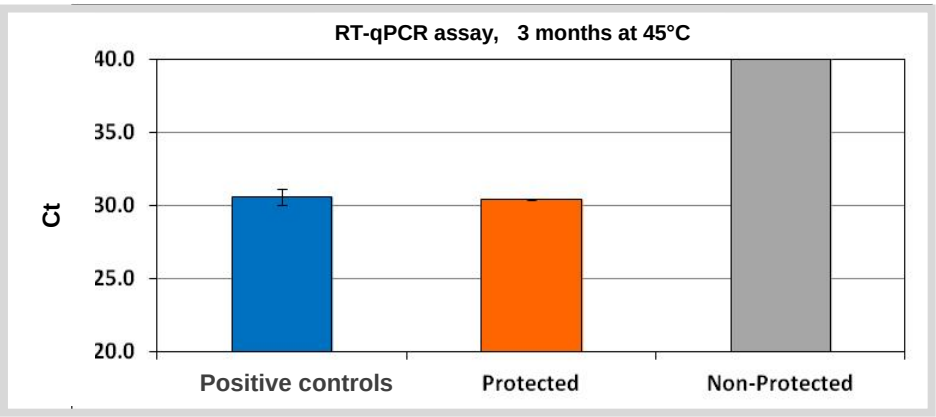

Figure 2. RT-qPCR assay with enzymes, dNTPs and assay buffer stabilized in a dried format by PCRstable technology. The reagents of a TaqMan probe-based RT-qPCR assay, including limited amounts of enzymes, dNTPs and assay buffer, were dried in the presence (Protected) or absence (Non-Protected), of PCRstable formulations, and were stored at 45°C for accelerated aging studies. After 3 months of storage at 45°C, the reagents were rehydrated with water; additionally, primers, TaqMan probes, and a known input of RNA template were added. The stabilities of the reagents preserved by the PCRstable formulations were assessed by reverse transcription and qPCR amplification. Reagents stored at -20°C were used as positive controls. Ct values show that the PCRstable formulations stabilize enzymes, dNTPs and assay buffer for more than 1 year at ambient temperatures based on the accelerated aging studies.

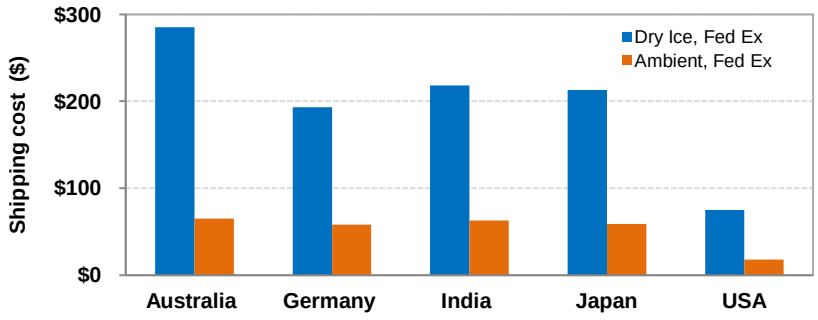

Figure 3. Comparison of shipping cost between dry ice shipping vs. ambient temperature shipping. 10 assay reagents were shipped with or without 10 lbs of dry ice by FedEx domestic shipping or international shipping (priority shipping for dry ice & standard shipping for ambient temperature). The results show that ambient temperature shipping significantly reduces the shipping cost.

Results and Discussion

PCR-based testing in regions where there is unreliable or non-existent access to cold storage makes the use of these tests difficult. A reliable and cost effective alternative to cold chain storage would therefore greatly improve implementation of these tests in such locations, as well as significantly decrease overall testing costs. Biomatrica developed PCRstable technology to facilitate ambient temperature preservation of PCR assay components, thereby eliminating cold chain requirements and their associated high costs. In this study, we have examined the stability and functionality of RT-qPCR reagents stabilized by our PCRstable technology. The results show that stabilization of RT-qPCR reagents in dried format (Figure 1, 2) with PCRstable formulations leads to high retention of both stability and activity at elevated temperatures. This technology can be applied to RT-qPCR-based assays thereby eliminating cold chain requirements in locations without access to reliable cold storage. Application of PCRstable technology also improves PCR reagent shelf life and reduces shipping costs (Figure 3). In addition, PCRstable technology allows a simplified workflow for stabilization of PCR reagents. It eliminates complicated and expensive infrastructure, and allows for easy manufacturing scale-up in most formats including multi-well plates, tubes and microfluidic chips.

***Note**: PCRstable™ is a trademark of Biomatrica. For ordering PCRstable services, please email to info@biomatrica or call 858-550-0308.*