PCRstable™: Chemical Stabilization of Reagents for All-Ambient Diagnostic Assays

V. Liberal, S. de los Rios, G. Dodson, P. Singhal and R. Muller

Biomatrica, Inc., 5627 Oberlin Drive, Suite 120, San Diego, CA 92121

Biomatrica® THE BIOSTABILITY COMPANY

Abstract

The need for cold or frozen storage of reagents for diagnostic testing makes the use of these tests difficult in regions where access to freezers is either non-existent or undependable. PCRstable is a reliable and cost effective alternative, developed to stabilize diagnostic test components dry at ambient temperatures. We have developed and tested the stability and functionality of stabilized PCR and RT-PCR reagents, which are described in this study. We combined the test reagents with proprietary biostability compounds and applied a simple air drying procedure for dry format. Following accelerated aging studies at elevated temperatures, we performed both PCR and RT-PCR reactions to assess the ability of the stabilized reagents to perform the desired reactions. For both PCR and RT-PCR assays, we have shown excellent stability at ambient temperatures for both end-point and qPCR. We demonstrate that stabilization of PCR and RT-PCR reagents with PCRstable compounds leads to high retention of both stability and activity at ambient and elevated temperatures. These procedures can be applied to PCR-based diagnostic assays to eliminate cold chain requirements and simplify testing in places with lack of access to reliable cold storage, as well as in applications that may benefit from cost effective shelf life and shipping approaches.

Introduction

Diagnostic 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 diagnostic tests in these locations, as well as significantly decrease overall testing costs. Biomatrica is using its core bio-stabilizing technology to develop ambient temperature stabilization of diagnostic test components, thereby eliminating cold chain requirements and the associated high cost. We have developed and tested the stability and functionality of dried-down, stabilized PCR and RT-PCR reagents described in this study.

Materials and Methods

We have evaluated several different assays, testing varying combinations of dried down reagents with increasing complexity. The specified reagents were combined with proprietary stabilizers, incubated in either dry or liquid format at high temperatures and tested at defined time points. The dried reaction mixtures were rehydrated with the appropriate components to complete the specified PCR or RT-PCR assay and the reactions were tested. Depending on the assay, either gel electrophoresis (for end-point PCR) or Ct and melt curve analysis (for qPCR) were performed to assess the ability of the dried reagents to perform their indicated functions. Each condition was run in triplicate and compared to a frozen positive control.

Results

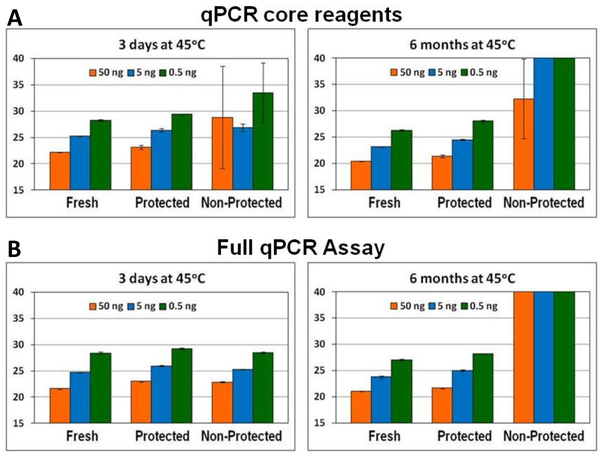

Figure 1. Stabilization of qPCR Assays. Core reagents for SYBR green qPCR assay, including Taq polymerase, dNTPs, primers and SYBR green dye (A), or complete qPCR assay, including reaction buffer (B), were dried down in the presence of Biomatrica's stabilizing formulations (Protected), or their absence (Non-Protected), and stored at room temperature or under stress conditions at 45°C. After the specified storage time, the assay components were rehydrated and their stability was assessed by qPCR amplification of a human RNase P amplicon, using a range of human genomic DNA input. Reagents stored frozen at -20°C (Fresh) were used as positive controls. Results show assay stabilization for more than 2 years at ambient temperatures (based on accelerated aging studies).

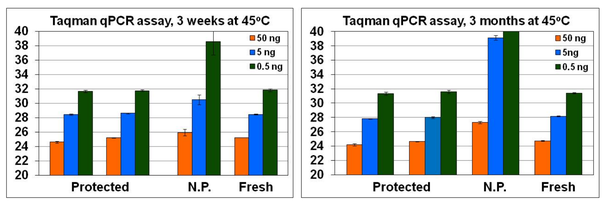

Figure 2. Stabilization of Full TaqMan qPCR Assay. A complete TaqMan probe-based qPCR assay, including reaction buffer, and TaqMan probes, was dried down in the presence of Biomatrica's stabilizing formulations (Protected), or their absence (Non-Protected, N.P.), and stored at room temperature or under stress conditions at 45°C. After the specified storage time, the assay was rehydrated and its stability was assessed by qPCR amplification of a human RNase P amplicon. Reagents stored frozen at -20°C (Fresh) were used as positive controls for the assay. Results show assay stabilization for more than 1 year at ambient temperatures (based on accelerated aging studies).

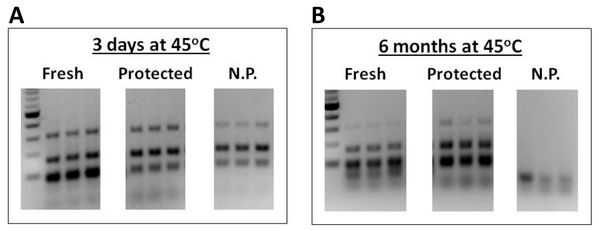

Figure 3: Stabilization of Reverse Transcriptase, Primers and dNTPs for Multiplex RT-PCR Assay. Reverse transcriptase, three sets of primers and dNTPs were dried down in the presence of Biomatrica's stabilizing formulations (Protected), or their absence (Non-Protected), and stored under stress conditions at 45°C. After the specified storage times, the reagents were rehydrated and their stability was assessed by reverse transcription followed by multiplex PCR amplification of 3 human TATA binding protein amplicons, using human RNA as template. Reagents stored frozen at -20°C (Fresh) were used as positive controls for the assay. Results show reagent stabilization for more than 2 years at ambient temperatures (based on accelerated aging studies).

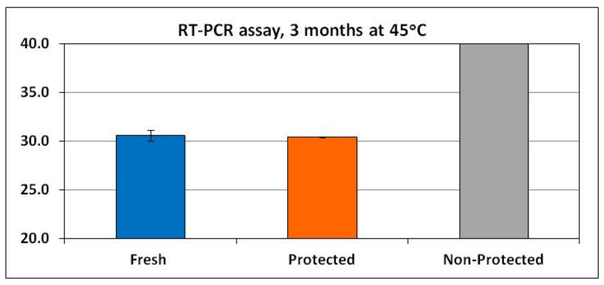

Figure 4. Stabilization of Complete RT-qPCR Assay. A TaqMan probe-based RT-qPCR assay, including limiting enzyme amounts, dNTPs and assay buffer, was dried down in the presence of Biomatrica's stabilizing formulations (Protected) or their absence (Non-Protected), and stored under stress conditions at 45°C. After 3 months of storage at 45ºC, samples were rehydrated with water, primers and TaqMan probes, and a known input of RNA template was added. Reverse transcription and real time PCR amplification were performed with equivalent amounts of template RNA samples, using RT-qPCR reagents stored at -20°C (Fresh) as a positive control. Results show assay stabilization for more than 1 year at ambient temperatures (based on accelerated aging studies).

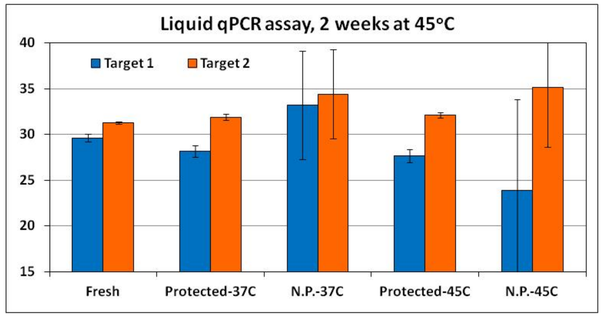

Figure 5. Stabilization of qPCR Assay In Liquid Format. A complete qPCR assay, including enzyme, dNTPs, primers/probes and reaction buffer, was stored in liquid format, in the presence of Biomatrica's stabilizing formulations (Protected), or their absence (Non-Protected, N.P.) at 37°C or 45°C. After the specified storage time, sample was added and the stability of the assay was assessed by qPCR. Reagents stored frozen at -20°C (Fresh) were used as positive controls. Results show assay stabilization for more than 1 year at ambient temperatures (based on accelerated aging studies).

Figure 6. Biomatrica's Easy Workflow for Dry Ambient Temperature Assay Stabilization. Biomatrica's robust PCRstable technology allows for an easy workflow for ambient assay stabilization. It eliminates complex and expensive infrastructures (A), and allows for easy manufacturing scale-up. Assay stabilization can be achieved in most formats, including multi-well plates, tubes and microfluidic chips (B).

Conclusions

- **Biomatrica's PCRstable technology stabilizes PCR and RT-PCR assays at ambient temperatures for extended periods of time (Figs. 1 - 5).**
- **Complete qPCR assays are stabilized for long-term storage at ambient temperatures (Figs. 1, 2 and 5).**
- **RT-PCR assays are stabilized for long-term storage at ambient temperatures (Figs. 3 and 4).**
- **Biomatrica's PCRstable technology allows for a simple and inexpensive workflow that can be applied to most assay formats (Fig. 6).**