



Stability of Custom Ready-To-Go products at ambient temperature for extended storage periods

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Abstract

We demonstrate the long-term ambient temperature stability of Custom Ready-to-Go products for molecular biology techniques, such as PCR, RT-PCR, whole genome amplification, and Western blotting. We created beads and cakes in a glassy state that exhibited glass transition temperatures above 65°C. These Ready-To-Go stabilized products performed the same after years of storage at ambient temperatures as they did before stabilization.

Introduction

GE Healthcare Life Sciences' patented Ready-To-Go technology is based on a proprietary mixture of excipients combined with lyophilization to provide long-term room temperature stability to biomolecules. The stability is attributed to the fact that the lyophilized proteins exist in an amorphous glassy state that markedly reduces molecular mobility and subsequent reactivity.

The glass transition temperature (T_g) for a lyophilized product indicates the temperature at which the glass state begins to transition to a more mobile "rubbery" state, allowing molecular mobility to return and a consequential loss of stability. Knowledge of the T_g value is thus important, as a lyophilized product must be stored below this temperature for long-term stability. All Ready-To-Go products from GE Healthcare exhibit T_g values well above room temperature, which ensures that the products can be shipped and stored without the need for refrigeration.

Since the introduction of the first Ready-To-Go product in 1992, the technology has demonstrated numerous benefits for the stabilization of a variety of reagents and complex



Fig 1. Amplified view of Ready-To-Go cakes, which can be formulated to provide ambient temperature stable, single-dose reaction mixtures.

assay mixtures. The proprietary technology is suitable for use with sensitive enzymes, antibodies, primers, probes, dyes and other reagents. The physically stable, solid reagent cake (Fig 1) provides long term stability at ambient temperature, can be dispensed in flexible formats, and is amenable to downstream manipulation.

The compatibility of Ready-To-Go technology with complex mixtures enables the formulation and stabilization of predispensed, single-dose reagents and reaction mixtures. This format can reduce cross-contamination risk and improve data reliability by requiring fewer pipetting steps and less handling overall. Fewer assay steps supports reduced training requirements and simplifies automation of the process.



The temperature stability of Ready-To-Go stabilized materials simplifies and reduces costs for shipping and storage. These materials do not require dry- or wet-ice shipment, which simplifies shipping across countries. Items can be shipped to remote regions or regions with insufficient infrastructure without the concerns or costs associated with temperature-controlled shipments. Once the materials arrive, they can be stored at ambient temperature at the point of use, in the field or lab.

GE Healthcare Life Sciences' Ready-To-Go stabilization service allows ambient temperature stabilization of customers' individual reagents and complete multiplex assays. The service is backed up by a dedicated custom team, including technical support, project management, supply chain management, and by over ten years of development and manufacturing experience. All Custom Ready-To-Go products are heat-sealed under low humidity conditions and stored at ambient temperature in an airtight foil pouch with desiccant.

To demonstrate the long term stability of Ready-To-Go formatted mixtures, the functional performance of various proteins and reaction mixtures was tested at the time of preparation and also after storage at ambient temperature for varying lengths of time.

Materials and methods

Formulation of Custom PuReTaq™ Ready-To-Go PCR cakes

Custom PuReTaq Ready-To-Go PCR cakes were formulated in a similar manner to illustra™ PuReTaq Ready-To-Go PCR Beads. The beads are premixed, predispensed, single-dose reactions optimized for performing standard PCR amplifications. The use of recombinant PuReTaq DNA Polymerase and other high purity reagents ensures extremely low levels of contaminating prokaryotic and eukaryotic nucleic acid, resulting in reliable and robust performance in both end point and real-time fluorescence-based PCR amplification.

Each ambient-temperature-stable cake contained stabilizers, buffer, dATP, dCTP, dGTP, dTTP, ~ 5 units of PuReTaq DNA Polymerase, and reaction buffer. To create the cakes, 20 µl was dispensed into a 96-well PCR plate and then lyophilized. When a cake is reconstituted to a final volume of 50 µl, the concentration of each dNTP is 200 µM in 10 mM Tris-HCl (pH 9.0), 50 mM KCl, and 1.5 mM MgCl₂.

Formulation of Custom Ready-To-Go RT-PCR cakes

Custom Ready-To-Go RT-PCR cakes were formulated in a similar manner to illustra Ready-To-Go RT-PCR Beads. The beads are stable at room temperature and designed for performing single-tube one-step RT-PCR. Each room-temperature-stable cake contained M-MuLV reverse transcriptase, RNase inhibitor, buffer, nucleotides, and Taq DNA Polymerase. To create the cakes, 10 µl was dispensed into a 96-well PCR plate and then lyophilized. When a cake is reconstituted to 50 µl, the concentration of each dNTP is 200 µM in 10 mM Tris-HCl pH 9.0, 60 mM KCl, and 1.5 mM MgCl₂.

Endpoint PCR analysis

The performance of Custom PuReTaq Ready-To-Go PCR cakes was tested using endpoint PCR amplification of the human p53 gene from human genomic DNA. Each reaction was prepared using one Custom PuReTaq Ready-To-Go PCR cake, (or two¹ illustra PuReTaq Ready-To-Go PCR beads as a control), 1 µl of each 10 µM forward and reverse p53 primers², 4 ng genomic DNA and water to a total volume of 50 µl. PCR amplifications were performed using a PTC-200 thermal cycler (MJ Research) using the following PCR cycling conditions: 95°C for 3 min, followed by 35 cycles of 95°C for 30 sec; 55°C for 1 min; 72°C for 1 min.

RT-PCR

The Custom Ready-To-Go RT-PCR cakes were tested using *Drosophila melanogaster* mRNA (Clontech Laboratories, Inc.) and gene-specific primers for a 425 bp sequence from the female sterile homeotic (fsh) gene. First strand cDNA synthesis was performed using one Custom Ready-To-Go RT-PCR cake, mRNA (0.2 ng) with pd(T)₁₂₋₁₈ primers (0.5 µg) or mRNA (1 ng) with pd(N)₆ primers (5 µg), and water to 50 µl volume for 15 min at 42°C, followed by 5 min at 95°C. After cooling to 4°C, gene-specific fsh primers³ (30 pmoles each) were added and amplification in a PTC-200 thermal cycler (MJ Research) used the following PCR cycling conditions: 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec; 68°C for 30 sec; 72°C for 1 min.

Reactions were analyzed using agarose gel electrophoresis with gels imaged using Typhoon™ Variable Mode Imager with ImageQuant™ software.

GenomiPhi™ amplification

Purified human genomic DNA (10 ng) was amplified using the standard protocol for the illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit. DNA yield was quantitated using Quant-iT™ PicoGreen™ dsDNA Reagent (Life Technologies Corp.) following the standard protocol. Briefly, amplification products were diluted 1:10 in TE buffer, then 5 µl volumes were added to 95 µl of TE in a plate, to which 100 µl of 1:25 diluted PicoGreen reagent was added. The fluorescence was recorded and DNA yields interpolated from a standard curve.

Results

Glass transition temperatures

Several Custom Ready-To-Go stabilized products were tested after prolonged storage at room temperature in air-tight foil pouches with desiccant. Glass transition temperatures after long term storage were still well above room temperature (Table 1).

¹ Two beads used for a 50 µl PCR reaction (usually 1 bead for a 25 µl reaction)

² p53 forward primer 5'-CCGTCAGTAGATTAC-3'; p53 reverse primer 5'-GTGTGGAGTATTGGATG-3'

³ dm-1 (fsh) primer 5'-GCCCGCCGCAATACGAACC-3'; dm-2 (fsh) primer 5'-CAGCGCCACCCGACGATGACT-3'

Table 1. Glass transition temperature for Custom Ready-to-Go products after various storage periods^a

Custom Ready-To-Go product	Storage time (mo) at ambient temperature	Glass transition temperature (°C)
PuReTaq PCR cake (for 50 µl reaction)	32	66
RT-PCR cake (for 50 µl reaction)	17	74
GenomiPhi V3 cake	6	87
Goat anti-mouse-Cy TM 3B conjugate	12	78
Sheep anti-Mouse-HRP conjugate	12	85

^a T_g values are the mean of triplicate determinations using a Perkin Elmer Diamond differential scanning calorimeter. The products were stored at room temperature in air-tight foil pouches with desiccant for the indicated times (months).

Custom PuReTaq Ready-To-Go PCR cakes

Performance of the Custom PuReTaq Ready-To-Go PCR cakes was evaluated after storage at ambient temperature for 32 mo. Performance of the cakes was not affected by long-term storage as determined by endpoint PCR analysis (Fig 2). Results were reproducible among batches before and after storage.

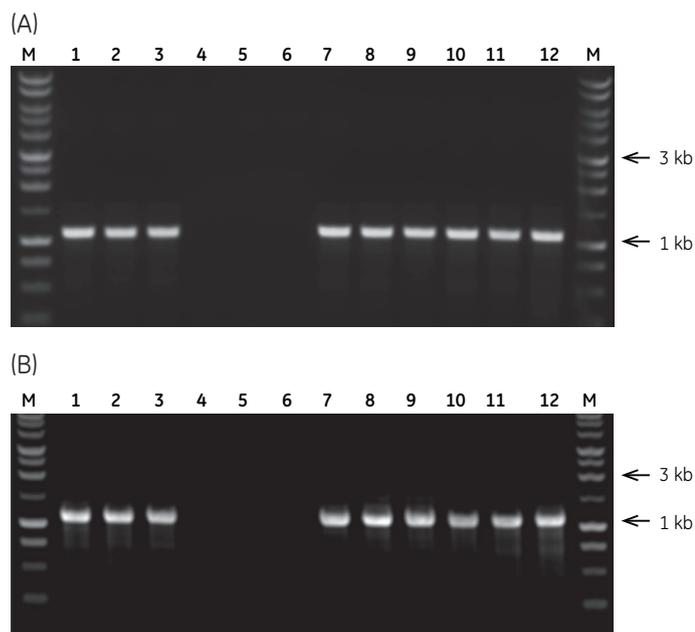


Fig 2. Agarose gel electrophoresis of PCR amplifications using Custom PuReTaq Ready-To-Go PCR cakes. The expected PCR product from the p53 specific primers is 1.1 kb. (A) Initial testing (T=0). Lanes 1 to 3: illustra PuReTaq Ready-To-Go PCR Beads; lanes 4 to 6: no template controls (NTCs); lanes 7 to 9: Custom PuReTaq Ready-To-Go cakes from batch #130809; lanes 10 to 12: Custom PuReTaq Ready-To-Go cakes from batch #290709; M = 1 kb DNA ladder. (B) Lanes 1 to 3: illustra PuReTaq Ready-To-Go PCR Beads; lanes 4 to 6: NTCs; lanes 7 to 9: Custom PuReTaq Ready-To-Go cakes from batch #130809; lanes 10 to 12 Custom PuReTaq Ready-To-Go cakes from batch #130809 after 32 mo of storage; M = 1 kb DNA ladder. Note: Only cakes from batch #130809 were tested 32 mo apart; other batches of cakes shown to indicate reproducibility of performance.

Custom Ready-To-Go RT-PCR cakes

Custom Ready-To-Go RT-PCR cakes retained their functional performance after ambient temperature storage for 17 mo. The cakes successfully amplified a 425 bp-fragment of the fsh gene of *D. melanogaster* (Fig 3). Performance was reproducible for mixtures containing either of the first-strand primers at the time points tested.

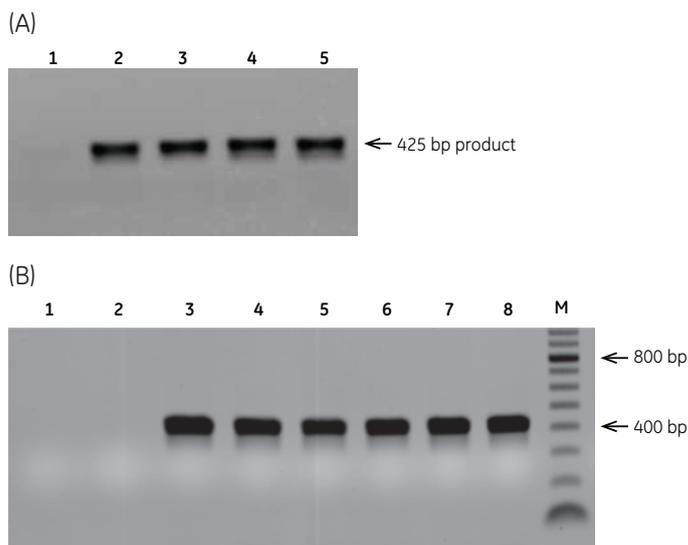


Fig 3. Agarose gel electrophoresis of RT-PCR amplifications using Custom Ready-To-Go RT-PCR cakes. The expected product from the *Drosophila melanogaster* fsh gene-specific primers is 425 bp. (A) Initial testing (T=0). Lane 1 NTC; lanes 2 to 3: Custom Ready-To-Go RT-PCR cakes with first-strand pd(T)₁₂₋₁₈ primers; lanes 4 to 5: Custom Ready-To-Go RT-PCR cakes with first-strand pd(N)₆ primers. (B) Retest after 17 mo. Lanes 1 to 2: NTCs; lanes 3 to 6 Custom Ready-To-Go RT-PCR cakes with first-strand pd(T)₁₂₋₁₈ primers; lanes 7 to 8: Custom Ready-To-Go RT-PCR cakes with first-strand pd(N)₆ primers; M = 100 Base-Pair Ladder (code no. 27-4007-01)

illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit

illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit offers highly efficient and representative whole-genome amplification with microgram yields from nanogram amounts of DNA sample. The preformulated, predispensed, single-dose, lyophilized cakes contain Phi29 DNA polymerase, random hexamer oligonucleotides, nucleotides, salts and buffers. A typical DNA yield of 12 to 20 µg DNA can be achieved in less than 2 h from only 10 ng of genomic DNA input.

Phi29 DNA polymerase is a temperature-sensitive enzyme that requires long-term storage at -80°C when in liquid form. Lyophilization to a glassy state, however, produces an ambient temperature stable cake that yields reproducible results after storage at room temperature for several months (Fig 4).

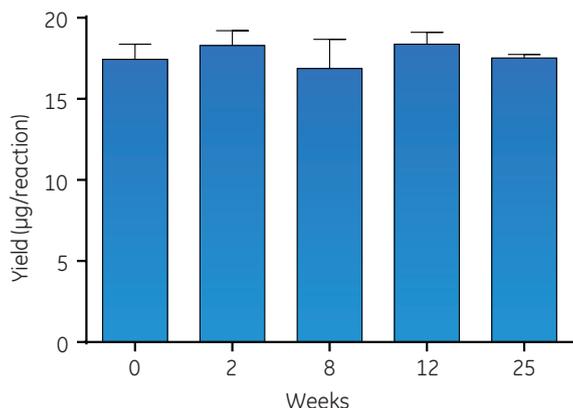


Fig 4. Yield of human genomic DNA amplified using illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit. DNA yield determined using Quant-iT PicoGreen dsDNA Reagent. Mean +/- SD of triplicate determinations.

Other Ready-To-Go stabilized custom products

A sheep anti-mouse-HRP conjugate was stabilized using Ready-To-Go technology and stored at ambient temperature for 1 yr. The Ready-To-Go product performed the same as the stock liquid conjugate (stored at 4°C) in both ELISA and Western Blotting applications (data not shown). Similarly, 1 yr ambient stability was seen with a goat anti-mouse-Cy3B conjugate (data not shown).

Conclusions

GE Healthcare Life Sciences' Ready-To-Go stabilization technology can be used to produce reagents and assay mixtures that are stable at ambient temperatures for years. The technology has been shown to be compatible with various nucleic acid amplification methods including standard PCR, RT-PCR, and whole genome amplification with Phi29 DNA polymerase. Antibody conjugates can also be stabilized into a Ready-To-Go format. Stabilized products perform the same after prolonged storage at ambient temperatures as they did before stabilization.

Ordering information

Product	Quantity	Code number
illustra PuReTaq Ready-To-Go PCR Beads (Multiwell Plate)	96 reactions	27-9557-01
illustra Ready-To-Go RT-PCR Beads (0.2 ml Tubes)	96 reactions	27-9267-01
illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit	96 reactions	25-6601-96

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Phi 29 DNA polymerase: For use only as licensed by Qiagen GmbH and GE Healthcare Bio-sciences Corp. The Phi 29 DNA polymerase enzyme may not be re-sold or used except in conjunction with the other components of the Ready-To-Go GenomiPhi V3. See U.S. patent Nos. 5,854,033, 6,124,120, 6,143,495,5,001,050, 5,198,543, 6,323,009, 5,576,204 and related U.S. and foreign patents.

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