HEMAgene™•BUFFY COAT

# Long-term stability of DNA from buffy coat samples stored in HEMAgene™•BUFFY COAT DNA stabilizing reagent

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*The long-term storage of buffy coat samples requires the use of ultra-low temperature freezers and temperature-monitoring devices, both of which are costly and unreliable in the event of a power failure. HEMAgene•BUFFY COAT (HG-BCD) DNA stabilizing reagent offers reliable, long-term room temperature stabilization of DNA in buffy coat samples. This document provides evidence that HG-BCD samples stored at room temperature maintain high molecular weight DNA for at least 3 years, as demonstrated by accelerated aging.*

## Introduction

Buffy coat fractions are often prepared from whole blood because they provide a clean, concentrated source of nucleated cells from which to extract DNA. Unlike whole blood, buffy coat preparations are more conducive to long-term storage and transport as long as they are kept frozen. Long-term storage of buffy coat samples requires significant capital and operational investment both in ultra-low temperature freezers and temperature monitoring alarm systems. Nonetheless, samples remain at risk in the event of a power failure. According to the McGill University Green Biobanking survey, 25% of respondents had experienced specimen loss as a result of power failures in the past 5 years, and 50% of respondents have experienced 1–20 power failures in the past 5 years that have resulted in relocation of specimens.[1]

An ideal solution to this problem is a biostabilizer that would enable long-term room temperature storage of buffy coat samples. HG-BCD for buffy coat samples offers reliable, long-term room temperature preservation of DNA in buffy coat samples. This technical paper provides evidence that HG-BCD effectively preserves high molecular weight DNA in buffy coat samples for at least 3 years at room temperature.

## Materials and methods

### *Sample collection and buffy coat preparation*

Three donors were recruited for this study and three blood draws per donor were made. For each of the three draws per donor, approximately 7 mL of whole blood was collected into a 10 mL EDTA-K Vacutainer tube. Samples were gently rocked at room temperature and then centrifuged at 1,200 × *g* for 10 minutes at room temperature to fractionate samples into plasma, buffy coat (5–10× concentrated leukocytes) and packed red blood cell (erythrocytes) fractions. Plasma was gently removed from the fractionated samples with a Pasteur pipette, leaving approximately 1 mL of plasma above the buffy coat layer. Using a P200 micropipette (set at 100 µL) and "wide-bore" pipette tips, a 0.5 mL aliquot of the buffy coat fraction was transferred to a 15 mL conical tube. 3 × 15 mL conical tubes containing 0.5 mL of buffy coat fractions were treated as follows for each donor:

**-80°C samples:** the 0.5 mL buffy coat sample was lysed and stabilized by adding 4.5 mL of HG-BCD to the sample, vortexed to ensure complete mixing and solubilization of the sample, apportioned into 0.5 mL aliquots in 1.5 mL micro-centrifuge tubes and stored at -80°C prior to extraction and analysis at pre-determined time points.

**RT samples:** the 0.5 mL buffy coat sample was lysed and stabilized by adding 4.5 mL of HG-BCD to the sample, vortexed to ensure complete mixing and solubilization of the sample and stored at room temperature (RT) prior to extraction and analysis at pre-determined time points.

**+50°C accelerated aging samples:** the 0.5 mL buffy coat sample was lysed and stabilized by adding 4.5 mL of HG-BCD to the sample, vortexed to ensure complete mixing and solubilization of the sample and stored at +50°C prior to extraction and analysis at pre-determined time points.

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### *DNA extraction*

Genomic DNA was purified from the Day 1 RT, +50°C and -80°C HG-BCD samples using DNA Genotek's recommended protocol for HG-BCD samples and the Promega ReliaPrep™ Blood gDNA Miniprep system.[2]

The formation of denatured protein aggregates is an expected phenomenon when storing whole blood or buffy coat samples at elevated temperatures for extended periods of time. These denatured proteins interfere with genomic DNA purification when using column based methodologies. As a result, genomic DNA was purified from the "35 week" RT, +50°C and -80°C HG-BCD samples using DNA Genotek's recommended protocol for HG-BCD samples and the Agencourt GenFind v2 Blood and Serum Genomic DNA Isolation Kit.[3]

### *Fluorometric determination of DNA concentration*

DNA yields from the HG-BCD samples were determined using Quant-iT™ PicoGreen® dsDNA Reagent (Invitrogen P7581) and a Lambda DNA Standard (Invitrogen, 25250010). PicoGreen is a fluorescent double-stranded DNA-binding dye (480 nm excitation/520 nm emission) that enables sensitive quantification of small amounts of double-stranded DNA. An aliquot of each sample was diluted 10-fold with TE buffer. Triplicate 5 µL aliquots of each sample and a standard curve of Lambda DNA (in triplicate; 0–10 ng/µL) were mixed with PicoGreen reagent in a black flat-bottomed 96-well microplate (Greiner Bio-One, 655209). Fluorescence was measured using an Infinite® M200 microplate reader (TECAN®).

### *Genomic DNA integrity*

To assess DNA integrity, 100 ng from each HG-BCD sample was separated on a 0.8% agarose gel by electrophoresis for 1 hour at 80 volts. The gel was stained in 1 µg/mL ethidium bromide in distilled water for 15 minutes at room temperature, rinsed and photographed on a UV transilluminator using a DigiDoc-IT™ imaging system (UVP LLC). The UltraRanger 1 kb DNA Ladder (300 bp–24,000 bp; Norgen Biotek) was used as a size reference for the genomic DNA samples.

### *DNA amplification*

Purified DNA was evaluated in qPCR for amplification performance using primers targeting the single copy thymidylate synthase gene (TYMS locus; NM001071.2). For each reaction, 50 ng of purified genomic DNA was amplified in a 25 µL volume containing: 1× PCR buffer (20 mM Tris, 50 mM KCl), 2 mM $MgCl_2$, 200 µM dNTPs (Invitrogen), 50 µg/mL BSA (Sigma Aldrich), 1 µM SYTO9 dye (Invitrogen), 0.4 µM each of Primer hTSm143F (GCCCTCTGCCAGTTCTA) and hTSm143R (TTCAGGCCCGTGATGT ), 1U Taq polymerase (Invitrogen). The amplification conditions for the TS143 target were: 1 cycle: 95°C for 5 minutes; 35 cycles: 95°C for 20 seconds, 55°C for 20 seconds, 72°C for 30 seconds and 1 cycle 72°C for 10 minutes. A melt curve program was included and consisted of: 1 cycle 95°C for 30 seconds at a ramp rate of 4.4°C/second (no acquisition), 72°C for 10 minutes at a ramp rate of 2.2°C/second (no acquisition), 95°C at a ramp rate of 0.11°C/second (continuous acquisition). DNA samples were run in triplicate in a Corbett Rotorgene RG-6000 and $C_t$ values for each sample calculated using the Rotorgene 6000 series software 1.7.

## Results

Analysis of the genomic DNA isolated from the RT, +50°C accelerated aging and -80°C HG-BCD samples by agarose gel electrophoresis (Figure 1) shows high molecular weight genomic DNA (>24,000 bp) in both the "day 1" and "35 week" samples and no evidence of degradation as a result of storage time or storage temperature between the "day 1" and "35 week" HG-BCD samples.


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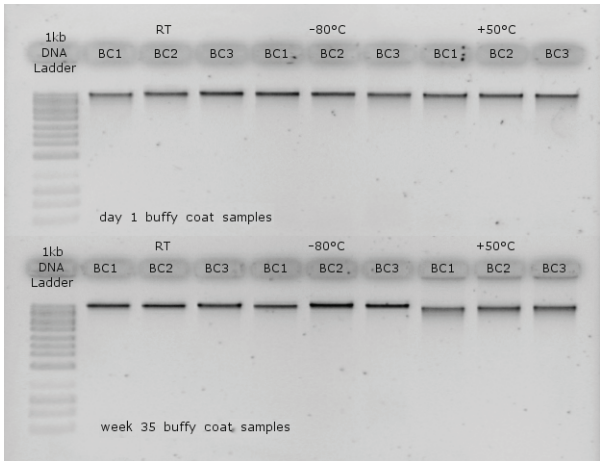


***Figure 1**: 200 µL aliquots of HG-BCD samples stored at room temperature (RT), +50°C and -80°C for the indicated time periods were used for genomic DNA isolation as described in Materials and methods. Aliquots (~100 ng) of the purified genomic DNA samples were analyzed by agarose gel electrophoresis as described in Materials and methods.*

The quantitative real time PCR (qPCR) results shown in Table 1 and Figure 2 demonstrate that the genomic DNA in each RT, +50°C accelerated aging and -80°C HG-BCD sample is free of contaminants or inhibitors and is suitable for downstream molecular biology applications, including qPCR.

| Sample treatment | HG-BCD sample | $C_t$ value | |
|---|---|---|---|
| | | Day 1 | Week 35 |
| RT | Donor 1 | 19.0 | 19.0 |
| | Donor 2 | 18.9 | 18.2 |
| | Donor 3 | 18.8 | 18.9 |
| +50°C | Donor 1 | 19.2 | 18.8 |
| | Donor 2 | 19.6 | 18.8 |
| | Donor 3 | 19.0 | 18.5 |
| -80°C | Donor 1 | 19.0 | 18.8 |
| | Donor 2 | 19.9 | 18.9 |
| | Donor 3 | 19.2 | 19.3 |

***Table 1**: 200 µL aliquots of RT, -80°C and +50°C HG-BCD samples were used for genomic DNA isolation as described in Materials and methods. Aliquots (~50 ng) of the eluted DNA samples were analyzed by qPCR on a Corbett Rotorgene RG-6000 as described in Materials and methods.*

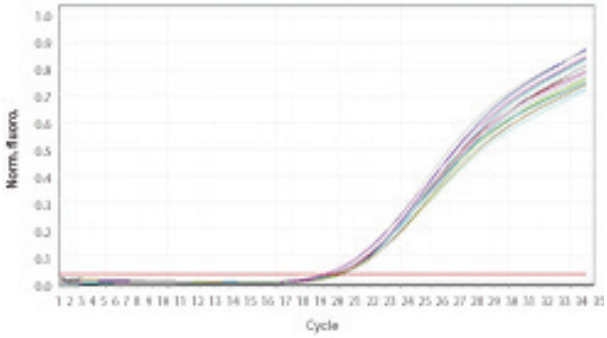


***Figure 2**: 200 µL aliquots of the RT, -80°C and +50°C HG-BCD samples were used for genomic DNA isolation using the Promega ReliaPrep gDNA Miniprep System (day 1) and the Agencourt GenFind v2™ Blood and Serum Genomic DNA Isolation Kit (week 35) as described in Materials and methods. Aliquots (~50 ng) of the eluted DNA samples were analyzed by qPCR on a Corbett Rotorgene RG-6000 as described in Materials and methods.*

## Discussion and conclusions

Agarose gel electrophoresis analysis of the purified RT, +50°C accelerated aging and -80°C HG-BCD samples showed high integrity and quality of the genomic DNA for all samples. The HG-BCD samples stored at +50°C showed evidence of protein denaturation, aggregation, clumping and precipitation as early as 3 weeks with an evident flocculant "pellet" at the bottom of the tube and clear, pinkish "supernatant". When these samples were resuspended, the insoluble protein "clumps" and aggregates prevented the direct purification of the genomic DNA using the Promega Reliaprep™ gDNA MiniPrep spin columns. Consequently, genomic DNA was purified from the "35 week" RT, +50°C and -80°C HG-BCD accelerated aging samples using the Agencourt GenFind v2™ Blood and Serum Genomic DNA Isolation Kit. No signs of significant DNA degradation or loss were observed in the +50°C HG-BCD samples.

Accelerated aging studies of HG-BCD samples stored at +50°C for 35 weeks indicated that the genomic DNA remains stable for at least 36 months (3 years) at room temperature (Figure 1). This was calculated using the Arrhenius equation and is based upon the assumption that the rate of a chemical reaction typically decreases by half for every 10°C decrease in temperature. Therefore the rate of chemical degradation of DNA at +24°C is expected to be 5-fold slower than the rate of degradation at +50°C.


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In addition, the TS143 qPCR product was amplified successfully from the purified HG-BCD genomic DNA samples, indicating the DNA is free of contaminants or inhibitors and is suitable for PCR analysis.

In summary, HG-BCD samples in appropriately sealed tubes (e.g., 0.5–2.0 mL screw cap tubes with O-rings to prevent evaporation) are expected to maintain the integrity of high molecular weight genomic DNA at room temperature for at least 3 years.

As this is an on-going accelerated aging study, this white paper will be updated with the results from subsequent time points.

**Reference:**

1 McCarthy, C. (2012). A survey on long-term storage of biological specimens at McGill University. http://www.medicine.mcgill.ca/pharma/Green/Documents/Survey%20Poster.pdf
2 Compatibility of the Promega ReliaPrep Blood gDNA Miniprep System with fresh buffy coat samples in HEMAgene•BUFFY COAT stabilizing reagent. DNA Genotek. MK-AN-00027.
3 Compatibility of the Agencourt GenFind v2 Blood and Serum Genomic DNA Isolation Kit with fresh buffy coat samples in HEMAgene•BUFFY COAT stabilizing reagent. DNA Genotek. MK-AN-00028.

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