HEMAgene™•BUFFY COAT (HG-BCD) Preservative Compatibility with ReliaPrep™ Large Volume HT gDNA Isolation System

Materials Required:

- ReliaPrep™ Large Volume HT gDNA Isolation System (Cat.# A2751)
- HSM 2.0 Instrument (Cat.# A2715)
- HEMAgene™•BUFFY COAT Preservative (HG-BCD, DNA Genotek)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *ReliaPrep™ Large Volume HT gDNA Isolation System Technical Manual,* #TM341.

HEMAgene™•BUFFY COAT (HG-BCD) preservative is a DNA stabilizing reagent designed for ambient temperature transport and room-temperature storage of buffy coat and white blood cell pellets derived from whole blood (1). The ability to transport and store samples at ambient temperature reduces the high cost of cold temperature handling and freezer storage, while lowering the risks of sample degradation. The reagent can be used with fresh or frozen samples and is able to withstand multiple freeze-thaw cycles (2). In this report, we examine the compatibility of buffy coat DNA preserved in HEMAgene™•BUFFY COAT (HG-BCD) reagent with DNA isolated using the ReliaPrep™ Large Volume HT gDNA Isolation System.

Methods

Whole blood from 3 individuals was collected in K_2 EDTA Vacutainer® tubes (BD Biosciences). The samples were centrifuged at 2,000 x *g* for 20 minutes to collect a buffy coat. A buffy coat consisting of 10% of the total volume of whole blood per tube was then removed (e.g., 1ml of buffy coat from 10ml of whole blood). Buffy coats from the same individual were pooled and preserved in HEMAgene™•BUFFY COAT (HG-BCD) Reagent as follows:

1. Transfer 2ml of buffy coat to a 50ml conical tube.
2. Add 18ml of HG-BCD reagent to the sample and mix vigorously by vortexing for 15 seconds.
3. Store the sample at room temperature for five days.

DNA from buffy coat preserved in HG-BCD was purified using the ReliaPrep™ Large Volume HT gDNA Isolation System in triplicate. DNA purification was performed manually on the HSM 2.0 Instrument according to the *ReliaPrep™ Large Volume HT gDNA Isolation System Technical Manual,* #TM341 as follows:

4. Add 3ml of HG-BCD sample to 50ml conical tubes in triplicate.
5. Add 60µl of Proteinase K to a tube.
6. Add 375µl of Alkaline protease to each tube. Shake at 500rpm for 1 minute.
7. Add 3ml of Lysis Buffer to each sample. Incubate at 65°C for 30 minutes with shaking at 550rpm.
8. After incubation, shake samples at 550rpm for an additional 10 minutes.

9. Add 3.6ml of Binding Buffer to each sample and shake for 3 minutes at 550rpm.
10. Thoroughly resuspend ReliaPrep™ Resin and add 300µl to each sample. Mix the resin and sample for 20 minutes at 550rpm.
11. Collect the resin for 20 minutes. Slowly aspirate the liquid from the tube, leaving only the collected resin.
12. Resuspend the resin in 5ml of Prepared Wash Buffer A. Mix samples for 2 minutes at 550rpm. Then thoroughly mix the resin with the Prepared Wash Buffer by pipetting the sample 10 times.
13 Mix samples at 500rpm for 2 minutes and collect the resin.
14. Aspirate wash buffer from the tubes and add another 5ml of Prepared Wash Buffer. Mix samples at 500rpm for 3 minutes, then at 700rpm for an additional 3 minutes.
15. Collect resin again, then aspirate the wash buffer.
16. Perform a final wash with 4ml of 50% Ethanol. Mix for 4 minutes at 500rpm.
17. Aspirate liquid from the sample and 1.5ml of Nuclease-Free Water to elute the DNA. Mix the water and resin for 3 minutes at 600rpm, followed by mixing at 400rpm, 70°C for 20 minutes.
18. Collect the resin and remove the liquid from the sample. Dispense into an intermediate 1.5ml microcentrifuge tube.
19 Centrifuge the tubes at maximum speed for 1 minute to pellet any carryover resin. Transfer the DNA to a clean 1.5ml tube for use.
20. Quantify the purified DNA by UV-absorbance using the NanoDrop®-1000 and by fluorescence dye detection using the QuantiFluor® dsDNA System and the GloMax® Discover plate reader.
21. Analyze DNA by gel electrophoresis on a 1% agarose gel to visualize DNA quality.
22. Perform qPCR using human-specific primers (GAPDH) to verify the amplifiability of the purified DNA.

Results

DNA was successfully purified from HG-BCD samples from all three individuals. DNA yields differed across the individuals (Table 1); however, the amount of DNA available will vary for each individual based on white blood cell count. The DNA yield in Table 1 is representative of a 3ml aliquot of the HG-BCD samples. The total expected yield from processing of the entire HG-BCD samples generated from a full 10ml tube of whole blood (3.3–3ml aliquots) ranges from 191.1–226.4µg of DNA for these individuals. All samples exhibited high purity based on absorbance ratios.

Table 1. DNA from 3ml of HG-BCD sample purified using the ReliaPrep™ Large Volume HT gDNA Isolation System. Concentration and yield were determined using the QuantiFluor® dsDNA System read on the GloMax® Discover System multimode reader. Purity measurements were determined using the NanoDrop®-1000. Values are an average of triplicate samples; standard deviations are listed.

Sample	Concentration (ng/µl)	Total Yield (µg)	A_{260}/A_{280}	A_{260}/A_{230}
1	61.5 ± 7.7	57.9± 6.8	1.84 ± 0.04	1.79 ± 0.06
2	66.0 ± 12.3	60.2 ± 10.7	1.89 ± 0.04	2.00 ± 0.00
3	73.0 ± 12.8	68.6 ± 9.8	1.92 ± 0.01	2.10 ± 0.02

Two-hundred nanograms (200ng) of purified genomic DNA from one replicate of each individual was separated on a 1% agarose gel (Figure 1). The DNA from all three individuals was of high molecular weight.

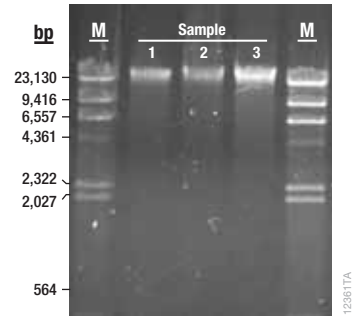
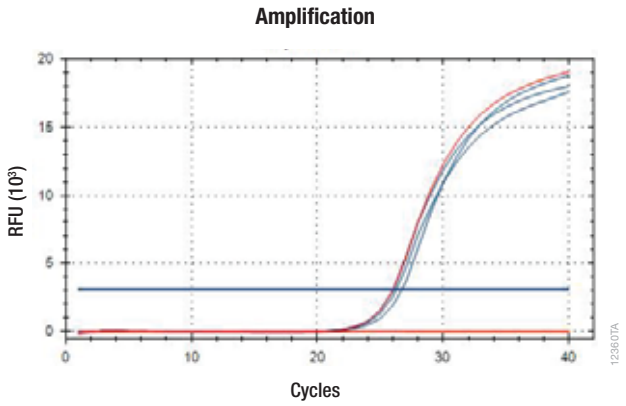


Figure 1. DNA from 3ml aliquots of HG-BCD sample was purified using the ReliaPrep™ Large Volume HT gDNA Isolation System. DNA (200ng) was loaded onto a 1% agarose gel and separated at 100V for 40 minutes. M = Lambda DNA/HindIII Markers (Cat.# G1711).

DNA purified from the HG-BCD sample was analyzed by qPCR using the GoTaq® qPCR System to verify amplifiability (Figure 2). GAPDH human-specific primers were used to amplify 50ng of DNA. C_q values for all three donors were consistent and similar to the control Human Genomic DNA (Cat.# G3041).



Sample	C_q Value
1	26.2
2	26.7
3	26.0
Control DNA	26.0
Average of three individuals	26.3

Figure 2. Fifty nanograms of DNA from each individual was used for quantitative real-time PCR analysis. C_q values were consistent for all samples (blue) and the control Human Genomic DNA (red).

Conclusions

Multiple freeze-thaw cycles can negatively impact the quality of DNA purified from stored whole blood and buffy coat samples. The HG-BCD reagent (DNA Genotek) provides an alternative to frozen storage by allowing for ambient temperature stabilization of high molecular weight DNA in fresh or frozen buffy coat samples (1,2). The ReliaPrep™ Large Volume HT gDNA Isolation System was used to successfully purify genomic DNA from buffy coat preserved in HG-BCD reagent after being stored for five days at room temperature. The purified DNA was of high molecular weight and purity, and amplifiable using the GoTaq® qPCR System without evidence of amplification inhibition.

References

1. PD-WP-00036: Long-term stability of DNA from buffy coat samples stored in HEMAgene™•BUFFY COAT DNA stabilizing reagent. http://www.dnagenotek.com/ROW/pdf/PD-WP-00036.pdf
2. PD-WP-00033: HEMAgene•BUFFY COAT DNA stabilizing reagent protects DNA in buffy coat samples through multiple freeze-thaw cycles. http://www.dnagenotek.com/ROW/pdf/PD-WP-00033.pdf