# HEMAgene™•BUFFY COAT (HG-BCD) Preservative Compatibility with ReliaPrep™ Blood gDNA Miniprep System

**Materials Required:**
- ReliaPrep™ Blood gDNA Miniprep System (Cat.# A5081, A5082)
- 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™ Blood gDNA Miniprep System Technical Manual,* #TM330.

HEMAgene™•BUFFY COAT (HG-BCD) 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 the ReliaPrep™ Blood gDNA Miniprep 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 vigorously vortex 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™ Blood gDNA Miniprep System in triplicate. The purification was done according to the *ReliaPrep™ Blood gDNA Miniprep System Technical Manual* #TM330 as follows:

4. Add 20µl of Proteinase K to a tube. Add 200µl of HG-BCD-preserved buffy coat to the tube, then add 200µl of Lysis Buffer. Mix the tube by vortexing for 10 seconds, and place in a heating block at 56°C for 10 minutes.
5. Add 250µl of Binding Buffer (BBA) to each sample and mix by vortex for 10 seconds.
6. Add the entire sample to a ReliaPrep™ Binding Column placed into an empty Collection Tube.
7. Centrifuge samples using an Eppendorf microcentrifuge 5418 R for 1 minute at maximum speed.

8. Discard the flowthrough and add 500µl of Column Wash Solution (CWD) to the ReliaPrep™ Binding Column.
9. Centrifuge the sample at maximum speed for 3 minutes and discard the flowthrough. Repeat this step two additional times for a total of three washes.
10. Place the ReliaPrep™ Binding column into a clean 1.5ml microcentrifuge tube and add 100µl of Nuclease-Free Water to the column. Centrifuge the samples at maximum speed for 1 minute to elute the DNA.
11. Quantify 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. Analyze DNA by gel electrophoresis on a 1% agarose gel to determine quality.
12. Perform qPCR using human-specific primers (GAPDH) to verify that the purified DNA could be amplified.

## Results

DNA was purified from HG-BCD samples from all three individuals. DNA yields differed across the three 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 200µl aliquot of the HG-BCD sample. The total expected yield from processing of the entire HG-BCD sample generated from a full 10ml tube of whole blood 50–200µl aliquots) would range from 120–220µg of DNA for these individuals. All samples exhibited high purity based on absorbance ratios.

**Table 1. DNA from 200µl of HG-BCD sample purified using the ReliaPrep™ Blood gDNA Miniprep System and eluted in 100µl.** 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 shown are an average of triplicate samples; standard deviations are listed.

| Sample | Concentration (ng/µl) | Total Yield (µg) | $A_{260}/A_{280}$ | $A_{260}/A_{280}$ |
|---|---|---|---|---|
| 1 | 23.8 ± 1.2 | 2.4 ± 0.1 | 1.84 ± 0.03 | 2.27 ± 0.06 |
| 2 | 37.6 ± 6.7 | 3.8 ± 0.7 | 1.88 ± 0.02 | 2.25 ± 0.02 |
| 3 | 43.8 ± 0.8 | 4.4 ± 0.8 | 1.94 ± 0.01 | 2.30 ± 0.06 |

To increase the concentration of genomic DNA purified with the ReliaPrep™ Blood gDNA Miniprep System, an additional test was done in triplicate on HG-BCD samples from individual 3. The ReliaPrep™ System protocol was performed as described above, except the DNA was eluted in 50µl instead of 100µl (Table 2). This increased the concentration of the gDNA recovered without significantly affecting the total yield.

**Table 2. DNA from 200µl aliquots of HG-BCD sample was purified using the ReliaPrep™ Blood gDNA Miniprep System and eluted in 50µl.** Concentration measurements were determined using the QuantiFluor® dsDNA System read on the GloMax® Discover plate reader. Purity measurements were determined using the NanoDrop®-1000. Values shown are an average of triplicate samples. Standard deviations are shown.

| Sample | Concentration (ng/µl) | Total Yield (µg) | $A_{260}/A_{280}$ | $A_{260}/A_{230}$ |
|---|---|---|---|---|
| 1 | 89.3 | 4.2 | 1.93 | 2.28 |
| 2 | 69.5 | 3.5 | 1.93 | 2.18 |
| 3 | 84.0 | 4.2 | 1.95 | 2.32 |
| Average | 79.1 ± 6.8 | 4.0 ± 0.3 | 1.94 ± 0.01 | 2.26 ± 0.06 |

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

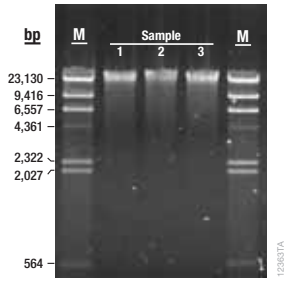


**Figure 1. DNA from 200µl aliquots of HG-BCD sample was purified using the ReliaPrep™ Blood gDNA Miniprep System.** gDNA (200ng) from each individual was loaded onto a 1% agarose gel and run at 100V for 40 minutes. M = Lambda DNA/HindIII Markers (Cat.# G1711).

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

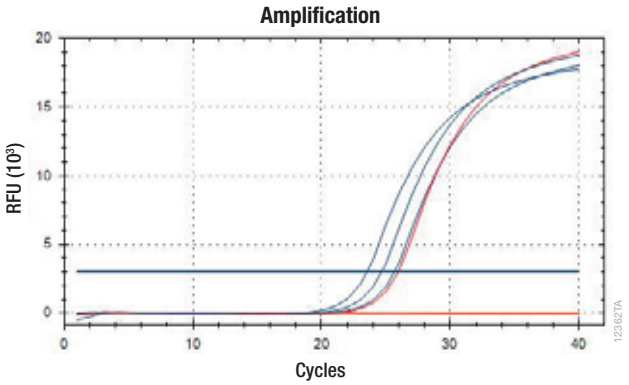


**Figure 2. Fifty nanograms of DNA from each individual was used for quantitative real-time PCR analysis.** $C_q$ values were consistent for all samples (blue) and the control Human Genomic DNA (red).

## 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 from 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™ Blood gDNA Miniprep 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. Higher concentrations of DNA were possible by eluting in 50µl versus a standard 100µl elution volume, without a loss in DNA yield. 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