HEMAgene™•BUFFY COAT (HG-BCD) Preservative Compatibility with the Maxwell® 16 LEV Blood DNA Kit

Materials Required:

- Maxwell® 16 LEV Blood DNA Kit (Cat.# AS1290)
- HEMAgene™•BUFFY COAT Preservative (HG-BCD, DNA Genotek)
- Maxwell® 16 Instrument (Cat.# AS2000)

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

Protocol: *Maxwell® 16 LEV Blood DNA Kit and Maxwell® 16 Buccal Swab LEV DNA Purification Kit Technical Manual*, #TM333

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 costs 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 reagent with the Maxwell® 16 LEV Blood DNA Kit.

Methods

Whole blood from 3 individuals was collected in K_2EDTA Vacutainer® tubes (BD Biosciences). The samples were centrifuged at 2,000 *x g* for 20 minutes to collect the buffy coat. The buffy coat consisting of 10% of the total volume of whole blood per tube was 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. Store the sample at room temperature for five days.

DNA from buffy coat preserved in HG-BCD was purified using the Maxwell® 16 LEV Blood DNA Kit in triplicate. The purification was done according to the *Maxwell®16 LEV Blood DNA Kit and Maxwell® 16 Buccal Swab LEV DNA Purification Kit Technical Manual*, #TM333 as follows:

3. Add 30µl of Proteinase K to a tube. Add 300µl of HG-BCD-preserved buffy coat to the tube, followed by addition of 300µl of Lysis Buffer. Vortex the tube for 10 seconds, and then place in a heating block at 56°C for 20 minutes.
4. Transfer the sample to well 1 of the Maxwell® 16 LEV Blood cartridge and run on the Maxwell® Instrument using the LEV Blood protocol.
5. Elute DNA with 50µl of elution buffer.
6. Quantify the purified DNA by UV-absorbance with 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 visualize the DNA quality.
7. Perform qPCR using human-specific primers (GAPDH) to verify the amplifiability of the purified DNA.

Table 1. Aliquots (300µl) of HG-BCD sample were used with the Maxwell® 16 LEV Blood DNA Kit. Concentration and yield 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 given.

Sample	Concentration (ng/µl)	Total Yield (µg)	A_{260}/A_{280}	A_{260}/A_{280}
1	75.8 ± 20.5	3.0 ± 0.8	1.76 ± 0.02	1.99 ± 0.1
2	106.6 ± 5.8	4.0 ± 0.2	1.80 ± 0.01	2.05 ± 0.02
3	182.0 ± 90.2	6.5 ± 3.1	1.81 ± 0.0	2.14 ± 0.03

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 based on white blood cell count. The DNA yield in Table 1 is representative of a 300µl aliquot of the HG-BCD samples. The total expected yield from the entire HG-BCD sample generated from a full 10ml tube of whole blood (33.3–300µl aliquots) would range from 99.9–216.5µg of DNA for these individuals. All samples exhibited high purity based on absorbance ratios.

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 samples was of high molecular weight.

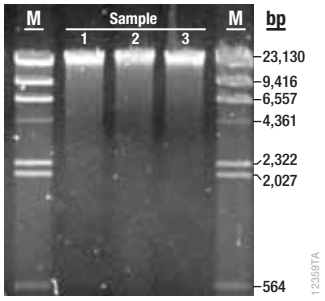
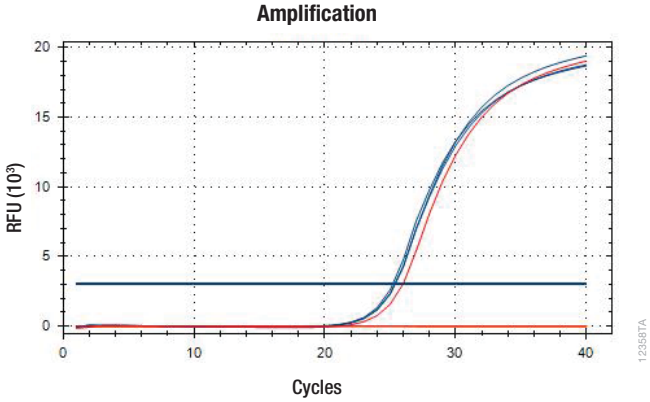


Figure 1. DNA from 300µl aliquots of HG-BCD sample was purified using the Maxwell® 16 LEV Blood DNA Kit. Two-hundred nanograms of genomic DNA from each individual was loaded onto a 1% agarose gel and run at 100V for 40 minutes. M = Lambda DNA/HindIII Markers (Cat.# G1711).

DNA purified from the HG-BCD sample was analyzed by qPCR using the GoTaq® qPCR System to verify amplifiability (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).



Sample	C_q Value
1	25.4
2	25.2
3	25.3
Control DNA	26.0
Average of 3 individuals	25.3

Figure 2. Fifty nanograms of DNA from each individual was used for quantitative real-time PCR analysis. C_q values were consistent for all individuals (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 Maxwell® 16 LEV Blood DNA Kit 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.pdf: 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.pdf: 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

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