

# **APPLICATION NOTE**

# Automated purification of DNA from buffy coat samples using the BioRobot® EZ1 System

 Buffy coat samples provide a highly concentrated source of nucleated blood cells and are commonly used as starting material for many clinical research applications. This study describes the efficient purification of high-purity DNA from a range of buffy coat samples prepared from citrate phosphate dextran- (CPD) or EDTA-preserved blood using the BioRobot<sup>®</sup> EZ1 System.

# Introduction

The leukocyte concentration in buffy coat samples varies considerably depending on the number of leukocytes in the blood sample, the method used to prepare the buffy coat layer, and the efficiency of harvesting the buffy coat sample. Automated purification of nucleic acid from buffy coat samples must be sufficiently robust to accommodate this variability while providing high-purity DNA that performs well in sensitive downstream applications such as PCR of single-copy genes.

# Materials and methods

Genomic DNA was purified from buffy coat samples prepared from CPD- or EDTApreserved human whole blood. Buffy coat samples were prepared by harvesting the intermediate layer of blood samples concentrated by centrifugation. Fully automated purification was performed using 75–100 µl highly enriched buffy coat samples and 150–300 µl buffy coat samples with normal to low leukocyte counts. Purified DNA was eluted in 200 µl water. DNA yield ( $A_{260}$ ) and purity was quantified by absorbance ( $A_{260}/A_{280}$ ) corrected for background. The EZ1 DNA Buffy Coat Card was used in combination with the BioRobot EZ1 DNA Blood 350 µl Kit.

# Results

Average DNA purity was 1.86 (±0.02). Sample input volumes and DNA yields are presented (Table 1). DNA yield was representative of the number of white blood cells (WBC) processed (Figure 1), and purified DNA performed consistently in PCR (Figure 2).





# Table 1. Reproducible DNA yields obtained from different buffy coat sample volumes using the EZ1 DNA Buffy Coat Protocol

Buffy coat sample	Donor	Sample volume (µl)	Buffy coat WBC count (10°/ml)	Average <sup>‡</sup> DNA yield (µg)
Highly enriched*	А	75	-	12.1 ±0.9
	А	100	-	13.1 ±1.1
Normal WBC count <sup>†</sup>	В	150	27.1	14.0 ±0.5
	С	150	21.0	12.9 ±1.4
	D	150	21.3	13.4 ±0.2
	Е	150	20.3	12.8 ±0.6
	F	150	-	8.5 ±1.3
	G	150	17.9	10.9 ±0.6
	Н	150	10.7	8.2 ±0.2
	Ι	150	9.8	6.8 ±0.1
Low WBC count	Н	300	10.7	13.7 ±0.3
	I	300	9.8	13.2 ±0.2

\* Prepared by centrifuging (300 x g for 10 minutes) from a CPD-preserved blood bag, ~10x enrichment.

<sup>t</sup> Prepared by centrifuging 10 ml EDTA-preserved blood tubes at 300 x g for 10 minutes.

<sup>‡</sup> DNA yields represent averages of 6 replicates.

Note: The 300 µl protocol is not recommended for highly enriched or normal buffy coat samples.



#### Representative Yields of High-Purity DNA from Buffy Coat Samples

**Figure 1.** Each data point represents the average yield from 3 replicates.







**Figure 2.** Amplification of a 900 bp single-copy gene (MECL-1) using template DNA purified from 8 different buffy coat samples with low to medium WBC concentration. Total DNA elution volume was 200 µl and 5 µl DNA was used as template in 50 µl PCR. A 5 µl (10%) aliquot of each PCR was visualized using a 1.5% agarose gel. **M**: 100 bp DNA ladder (100 ng); +: positive control; -: negative control.

## **Conclusions**

Using the BioRobot EZ1 System:

- n Yields of high-quality DNA are representative of sample concentration
- n Purified DNA performs consistently in sensitive downstream applications such as PCR
- n Buffy coat samples yield up to 14 µg DNA

### Contact QIAGEN today and discover easy automation for reproducible DNA preps from buffy coat samples!

#### Ordering Information

Product	Contents	Cat. No.
BioRobot EZ1	Robotic workstation for easy, automated purification of nucleic acids	9000705
EZ1 DNA Blood 350 µl Kit* (48)	48 Reagent Cartridges (Blood 350 µl), 50 Disposable Tip Holders, 50 Disposable Tips, 50 Sample Tubes (2.0 ml), 50 Elution Tubes (1.5 ml)	951054
EZ1 DNA Buffy Coat Card	Pre-programmed card for BioRobot EZ1 DNA Buffy Coat 75 μl, 150 μl, and 300 μl Protocols	9015587

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at <u>www.qiagen.com</u> or can be requested from QIAGEN Technical Services or your local distributor.



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BioRobot Systems are not available in all countries, please inquire.

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