# Genomic DNA Isolation from blood collected on different anticoagulants: Citrate, EDTA & Heparin

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## INTRODUCTION

Blood is an excellent sample for diagnostic purposes. It is rich in protein and nucleic acid-based biomarkers that can be used to detect a panel of diseases long before any physical symptoms become apparent. Studies have shown that cancer biomarkers, such as differentially methylated target genes, are detectable in blood samples and have proven to be extremely sensitive and specific for given cancer types[1]. These biomarkers can be cell associated, such as those found in leukocytes[2], or cell-free, such as those found in plasma or serum samples. For this reason, blood is often the sample of choice for biomarker or diagnostic research.

Investigators utilizing blood in their research have unique needs based on their downstream applications. Blood can be collected on different anticoagulants including citrate, EDTA or heparin. The type of anticoagulant used can affect the results from blood DNA isolation kits, and ultimately influence the results of research-based or diagnostic tests associated with blood. Investigators must ensure that their blood DNA isolation method is flexible, i.e. it can work efficiently on isolating blood from the specific anticoagulant used in blood collection. A robust blood DNA isolation kit eliminates sample processing biases, and increases data reproducibility.

The purpose of this study is to determine if Norgen's Blood Genomic DNA Isolation Mini Kit (Cat# 46300) can efficiently isolate blood genomic DNA from blood collected on different anticoagulants (Citrate, EDTA, Heparin).

## MATERIALS AND METHODS

### Sample Collection

Six milliliters of blood was collected in EDTA tubes, while 2.7 milliliters was collected in citrate tubes, and 3 milliliters was collected in heparin tubes from the same healthy individual, by a trained professional. The samples were frozen at -70ºC until processed.

### Blood DNA Extraction

DNA was extracted from the thawed blood sample using Norgen's Blood Genomic DNA Isolation Mini Kit (Cat# 46300), as per the manufacturer's instruction. Briefly, Proteinase K was added to a microcentrifuge tube, followed by 20µL of blood. Next, 300µL of Lysis Solution was then added and the samples were vortexed and incubated at 55ºC for 10 minutes. Next, 250µL of ethanol was added to each sample, and samples were bound, washed and eluted as per the manufacturer's protocol.

### Spectrophotometry

Blood DNA quantity was measured using the UltraSpec 2100 Pro (Fisher Scientific). Twenty five µL of each DNA elution was diluted with 475 µL of nuclease-free water, and OD measurements were taken using the cuvette-based spectrophotometry method.

### Real-Time PCR

The purified DNA was then used as the template in a real-time TaqMan® PCR reaction. Briefly, 3 µL and 9 µL of isolated DNA was added to a real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007), 0.4 µL from a 25mM GAPDH primer pair mix and 0.2 µL of the TaqMan® probe. The volume was brought up to 20 µL using nuclease-free water. The PCR samples were amplified under the real-time program; 95℃ for 3 minutes for an initial denaturation, 40 cycles of 95℃ for 15 seconds for denaturation, 60℃ for annealing and extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

## RESULTS AND DISCUSSION

Blood is an excellent resource for research and diagnostic purposes. Systemic diseases can be detected through a simple blood test, and changes in DNA (both cellular and cell-free) can be detected from blood, giving rise to its high potential for screening for a panel of diseases.

The key to the success of any study utilizing blood is a reliable blood DNA isolation method that performs optimally from blood collected on any anticoagulant. One can determine the flexibility of a kit by performing DNA isolation on blood from the same donor but collected in tubes with different anticoagulants in order to see the kit robustness and consistency. In this study, DNA was isolated from blood collected from the same donor on three different anticoagulants: citrate, EDTA, and heparin, using Norgen's Blood Genomic DNA Isolation Mini Kit. Fifteen microliters of each 200 µL elution was then run on a 1X TAE 1.0% agarose gel to visually inspect the isolated gDNA (**Figure 1**). The three different anticoagulants demonstrated similar DNA yield and integrity when observed visually on the gel.

In order to determine the yield of DNA isolated from each blood sample collected on the different anticoagulants, samples were measured using spectrophotometric method **(Figure 2).** The highest DNA yield was obtained from blood collected on heparin, while the DNA yields from citrate and EDTA were very similar. The OD260/280 was similar between the three conditions and the OD260/230 is non-significantly higher in citrate than EDTA than heparin **(Figure 3).**

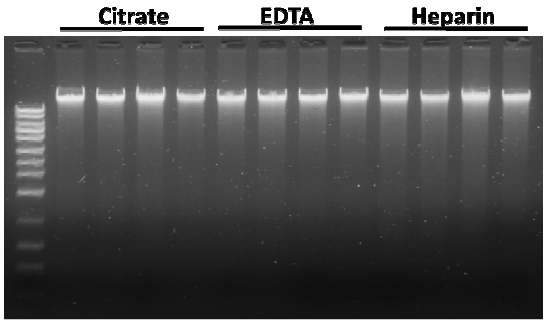


**Figure 1. Resolution of DNA isolated from blood collected on citrate, EDTA and heparin tubes and processed using Norgen's Blood Genomic DNA Isolation Mini Kit.** Fifteen microliters of each 200 µL elution was run on 1X TAE 1.0% agarose gel. Marker= Norgen's UltraRanger DNA Ladder.

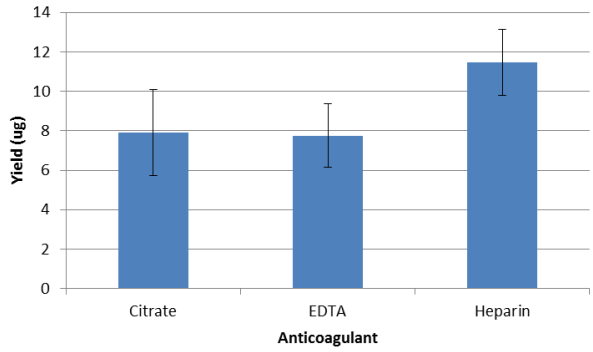


**Figure 2. The difference in DNA yield from blood collected on citrate, EDTA, and heparin anticoagulants and processed using Norgen's Blood Genomic DNA Isolation Mini Kit.** Twenty-five microliters of each sample was diluted in 475 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific). One elution was performed.

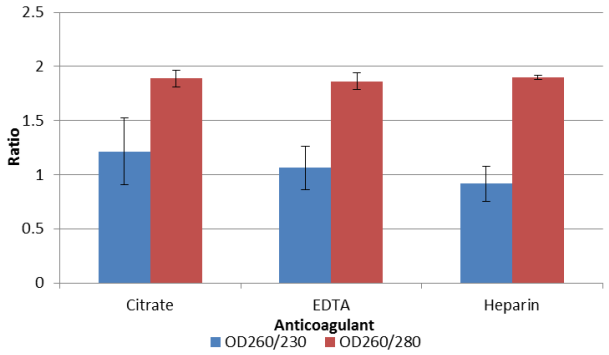


**Figure 3. OD260/280 and OD260/230 ratios of DNA isolated from blood collected on citrate, EDTA, and heparin anticoagulants and processed using Norgen's Blood Genomic DNA Isolation Mini Kit.** Twenty-five microliters of each sample was diluted in 475 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific). One elution was performed.

DNA quality was determined through the use of a TaqMan® Real-Time PCR method. In order to assess sample inhibition, 3 µL and 9 µL of each sample was used in the reaction. The Ct values were then graphed (**Figure 4**). It was found that qPCR performance was optimal with EDTA with the 3uL input, as seen by the lower Ct value. Similar Ct values were found for all with the 9uL input.

For all the blood samples processed, DNA isolated from all conditions consistently displayed a lower Ct value when increasing the DNA template amount from 3 µL to 9 µL.

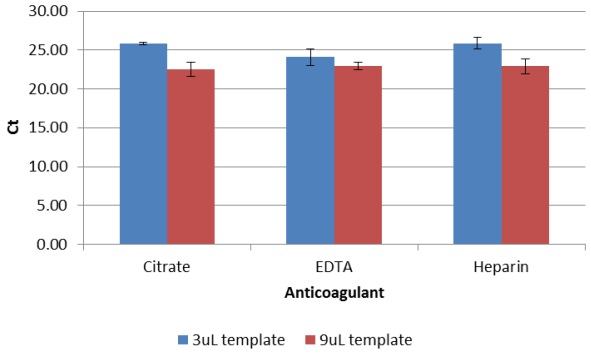


**Figure 4. The difference in Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated from blood collected on Citrate, EDTA, and Heparin anticoagulants.** Three and nine microliters of each elution were used in a 20µL qPCR reaction involving GAPDH primers.

## CONCLUSIONS

Norgen's Blood Genomic DNA Isolation Mini Kit can be used to isolate DNA from blood collected on citrate, EDTA or heparin, the 3 most commonly used anticoagulants. The highest yield was obtained from blood collected on heparin, and a similar DNA integrity, purity and qPCR performance was observed from the three anticoagulants.

## REFERENCES

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2. Koestler DC, Marsit CJ, Christensen BC, Accomando W, Langevin SM, Houseman EA, et al. 2012. Peripheral blood immune cell methylation profiles are associated with nonhematopoietic cancers. Cancer Epidemiol Biomarkers Prev; 21(8):1293-302.

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