



Whole blood DNA extraction from PAXgene™ Blood DNA Tube using GenFind V3

*This method is applicable to scientist that need to extract whole blood collected and stored in PAXgene™ Blood DNA tubes.
Please reference current GenFind V3 IFU for product information (Part Number: C34880, C34881)*

Purpose

The extraction of DNA from whole blood samples is critical for disease prediction and diagnosis. The PAXgene™ Blood DNA Tube is intended for the collection and stabilization of whole blood for genomic DNA (gDNA) isolation. Typically, before gDNA purification, scientists need to centrifuge the whole blood specimens to pellet down the cells and remove the liquid. This centrifuge step can be labor intensive and increase hands-on time. Here we show that the GenFind V3 protocol can process whole blood directly from PAXgene™ Blood DNA Tubes with no centrifugation step necessary.

Materials Used

Material	Part Number	Supplier
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
Nuclease-free water (Molecular Grade)	W3513	Sigma-Aldrich
2 ml 96-well plate	609681	Beckman Coulter
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
37°C heat block or water bath	N/A	N/A

Protocol

1. Lysis

- Mix** by **inverting** the PAXgene™ Blood DNA Tube 10 times
- Transfer **200 µL** of **sample** to a 2mL 96-well plate
- Add **500 µL** of **Lysis (LBB)** to plate
- Mix** by pipetting up and down 10 times, or until thoroughly mixed.
- Incubate** the plate for **10 minutes** at **37°C**

2. Bind

- Vortex the bottle of **Bind (BBB)** to fully resuspend the beads
- Add **300 µL** of **Bind (BBB)** to the plate
- Incubate** the plate for **5 minutes** at **room temperature**
- Place the plate on a **magnet** for **15 minutes** (or until supernatant is clear)
- Remove and discard the supernatant without disrupting the beads
- Remove the plate from the magnet

3. Wash

- a. Add **800 μ L** of **Wash (WBB)** to plate
- b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
- c. Place the plate on a magnet for 10 minutes (or until supernatant is clear)
- d. Remove and discard the supernatant without disrupting the beads
- e. Remove the plate from the magnet
- f. Repeat steps 3.a-3.e for a total of **2 washes**
- g. Add **1.6 mL** of **Wash (WBC)** to plate
- h. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
- i. Place the plate on a magnet for **10 minutes** (or until supernatant is clear)
- j. Remove and discard the supernatant without disrupting the beads
- k. Remove the plate from the magnet
- l. Repeat steps 3.g-3.k for a total of **2 washes**

4. Elute

- a. Add **200 μ L** of **nuclease-free water** to plate
- b. **Incubate** the plate for **2 minutes** at **room temperature**
- c. Place the plate on a **magnet** for **2 minutes** (or until supernatant is clear)
- d. Remove and **Save** the supernatant without disrupting the beads

Example Data

Genomic DNA extraction from blood collected in PaxGene DNA tubes

Genomic DNA (gDNA) was extracted from blood collected in PaxGene DNA tubes that were stored at 4°C for 12 days. The DNA concentration and purity were evaluated using a NanoDrop (Thermo Fisher Scientific). Three healthy donors were used to test the variability between different donors. Donors A and B had very similar DNA yields averaging 3.4 μ g with concentrations averaging 34.3 ng/ μ L ; while donor C averaged almost twice the DNA yield at 5.5 μ g with a concentration of 55.6 ng/ μ L. This difference in yield is most likely due to inherit biological variation among donors and not due to technical or kit variation. Although there is variation in the yield among the three donors, the purity as measured by A260/280 and A260/230 absorbance ratios was consistent between all three donors. The average A260/280 absorbance ratio was 1.8, and the average A260/230 absorbance ratio was 2.2, which are within acceptable ratios as indicated by ThermoScientific T042-Technical Bulletin.

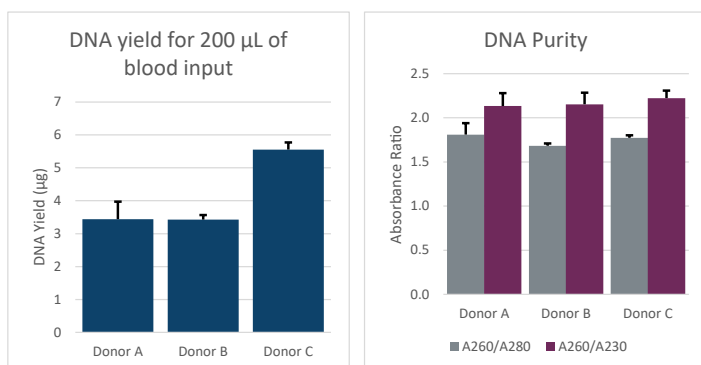


Figure. The average yield and purity from three technical replicates of three healthy donors as assessed using the NanoDrop (Thermo Fisher Scientific). Error bars are calculated by the standard deviation of three technical replicates.

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