Technical Note

DNA Purification from Buffy Coat Samples with the HigherPurity™ Blood Genomic DNA Extraction Kit

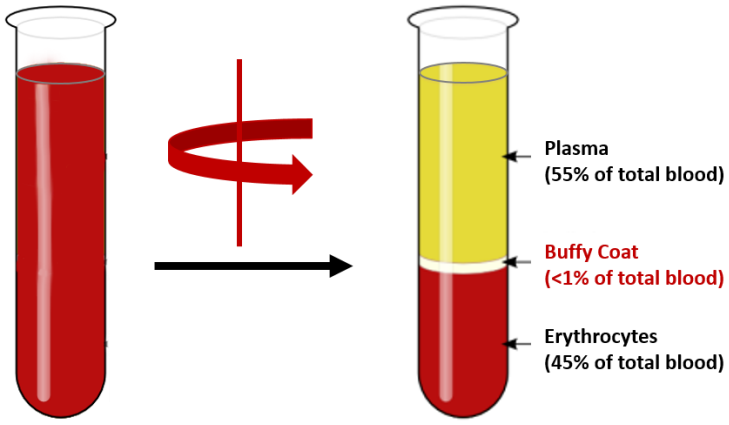
A buffy coat suspension is a concentrated leukocyte suspension. It is not mononuclear as the granulocytes are still present.

It is possible to concentrate the leukocytes from blood samples by centrifuging whole blood that has been anticoagulated with EDTA. After centrifugation, three different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes.

The use buffy coat allows to purify large amounts of gDNA using only very small samples. Buffy coat fraction from whole blood yields approximately 5–10 times more DNA than an equivalent volume of whole blood.

In this technical note, we describe the use of up to 1 ml of buffy coat per sample. This sample size corresponds approx. to 10 ml of whole blood sample.

Prepare buffy coat by centrifuging whole blood at 2500 x g for 10 minutes at room temperature (15–25°C).



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DETAILED PROTOCOL

Generally, the volume of buffy coat prepared from a sample is approximately 1/10 the volume of the original blood sample (e.g., 500µl of buffy coat would be prepared from a 5ml blood sample). Ensure that the buffy coat is well mixed before processing.

This protocol is for purification of genomic DNA from buffy coat prepared from a 5 ml whole blood.

1. Transfer up to **500 µl** of sample to a 15 ml tube.
2. Add **3V buffer S1** (e.g., if processing 500 µl buffy coat, dispense 1500 µl S1 buffer) to the sample and vortex gently or invert tube 6-8 times and leave to incubate for 10 minutes at room temperature.
3. Centrifuge at 2000xg for 5 minutes. Remove the supernatant using a pipette and avoiding damaging the cell visible pellet and leaving 200 µl of residual liquid.
4. Vortex the tube vigorously to resuspend the pellet in the residual liquid (10–15 seconds). **This process will help to optimize the cell lysis in the following step.**
5. Add **250 µl proteinase K** and **5 ml S2 buffer** and mixing by pipetting. Transfer all the mix into a new 15 ml tube.
6. Incubate in a water bath at 55 °C for 0.5-1 hour, and then cool to room temperature.
7. Add **1.67 ml S3 buffer** and mixing with vortex vigorously for 20 seconds.
8. Centrifuge at 2000xg for 5 minutes. A dark brown pellet should be visible. If no pellet is observed, incubate on ice for 5 minutes and centrifuge again.
9. Transfer the supernatant to a new 15 ml tube containing **5 ml isopropanol**. Mix by gentle inversion 50 times.
10. Centrifuge at 2000xg for 3 minutes and remove the supernatant. The DNA will be visible as a small white pellet.
11. Remove the supernatant using a pipette and dry the pellet with the tube inverted on absorbent paper, taking care that the pellet remains in the tube.
12. Wash with **5 ml 70% ethanol** and centrifuge at 2000xg for 1 minutes.
13. Remove the supernatant using a pipette and dry the pellet with the tube inverted on absorbent paper for 5-10 minutes.
14. Add **500 µL** of **Buffer EB** and vortex for 5 seconds at medium speed to mix. Close the cap and incubate for 1 hour.
15. Resuspend the DNA and store at -20 ° C.

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