gDNA Extraction from Blood Spots with Genfind v2

Blood can be spotted on filter paper or FTA cards for ease of transport and storage, and DNA can later be isolated from the spots.

Please reference the current Genfind v2 protocol for product information (Part Number A41497, A83078, A41499)

Purpose

Isolation of DNA from dried blood requires slight modification to the protocol for blood isolation. As the blood is on absorbent paper, additional lysis buffer is required. Optimal lysis conditions also differ slightly from those for whole blood.

Materials Used

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>PART NUMBER</th>
<th>SUPPLIER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genfind v2</td>
<td>A41497, A83078, A41499</td>
<td>Beckman Coulter</td>
</tr>
<tr>
<td>100% Ethanol (Molecular Grade)</td>
<td>AB00138</td>
<td>AmericanBio</td>
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<tr>
<td>Nuclease-free water (Molecular Grade)</td>
<td>AM9932</td>
<td>ThermoFisher Scientific</td>
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<tr>
<td>24 well, 10 mL Uniplate</td>
<td>WHA77015102</td>
<td>Whatman</td>
</tr>
<tr>
<td>903 Protein Saver Card</td>
<td>WHA10534612</td>
<td>Whatman</td>
</tr>
<tr>
<td>Magnum FLX 24 Magnet</td>
<td>A000270</td>
<td>Alpaqua</td>
</tr>
</tbody>
</table>

Protocol

1. Sample Preparation
   a. Blood spots are either cut out of the paper or punches are removed from the blood spots. This protocol can use up to two 0.5 inch circles containing 50 μL blood each. For smaller quantities of filter paper, lower amounts of lysis buffer and proteinase K can be used as long as the paper is covered in buffer.
   b. Add **600 μL Lysis LBC** and **18 μL of Proteinase K** to each sample
   c. Mix by pipetting up and down 10 times, or until thoroughly mixed

2. Lysis
   a. Incubate the sample either for **1 hr** at **room temperature** or for **30 min** at **37 °C**

3. Bind
   a. Vortex to fully resuspend the **Bind (BBB)**
   b. Add **300 μL of Bind (BBB)** to the plate
   c. **Incubate** the plate for **5 min** at **room temperature**
   d. Place the plate on the **magnet** for **15 min** (or until supernatant is clear)
   e. Remove and discard the supernatant without disrupting the beads
   f. Remove the plate from the magnet
4. Wash 1
   a. Add 800 μL of Wash (WBB) to plate
   b. Mix by pipetting up and down 10 times, or until thoroughly mixed
   c. Place the plate on the magnet for 10 min (or until supernatant is clear)
   d. Remove and discard the supernatant without disrupting the beads
   e. Remove the plate from the magnet
   f. Repeat step 4 for a total of 2 washes

5. Wash 2
   a. Add 500 μL of Wash (WBC) to plate
   b. Mix by pipetting up and down 10 times, or until thoroughly mixed
   c. Place the plate on the magnet for 6 min (or until supernatant is clear)
   d. Remove and discard the supernatant without disrupting the beads
   e. Remove the plate from the magnet
   f. Repeat step 5 for a total of 2 washes

6. Elute
   a. Add 80 μL of nuclease free water to plate
   b. Mix by pipetting up and down 10 times, or until thoroughly mixed
   c. Incubate the plate for 2 min at room temperature
   d. Place the plate on the magnet for 2 min (or until supernatant is clear)
   e. Remove and Save the supernatant without disrupting the beads
Example Data

Blood (50 μL per circle) was spotted onto Whatman 903 Protein Saver Cards (Cat# WHA10534612), and blood spots were dried at least overnight. Circles were then cut out with scissors, and two circles were used per sample. All blood spots were from a single blood donor.

DNA was extracted using the protocol shown above, with several different lysis conditions. Lysis was done for an hour at room temperature with 2000 rpm shaking or 30 min at 37 °C with 2000 rpm shaking. Conditions were done in triplicate. Yield was measured both by nanodrop and with Quant-it Picogreen dsDNA dye.

Both lysis methods tested resulted in greater than 650 ng of DNA per 100 μL of initial blood sample. Lysis for 30 min at 37 °C resulted in higher yield; however, room temperature lysis also works well if that is required for a particular workflow. Additional lysis time does not increase yield at 37 °C. Removal of PCR inhibitors was tested with qPCR, and both lysis methods appear to remove PCR inhibitors equally well (Figure 2), as seen by the similar Ct values. Both methods result in good yields of purified DNA and produce good results in qPCR.

![Figure 1](image1.png)
Figure 1. Yield of DNA from bloodspots with varying lysis conditions. Yield was measured by picogreen fluorescence and error bars represent the standard deviation of three technical replicates.

![Figure 2](image2.png)
Figure 2. Data from qPCR of bloodspot DNA (100 ng) with ActB primers. Both lysis conditions remove PCR inhibitors in equal amounts. Error bars represent the standard deviation of three technical replicates.

<table>
<thead>
<tr>
<th>SAMPLE ID</th>
<th>260/280</th>
<th>YIELD (NG, PICOGREEN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT 1 hr</td>
<td>2.01</td>
<td>803.3</td>
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<tr>
<td>RT 1 hr</td>
<td>1.89</td>
<td>651.4</td>
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<tr>
<td>RT 1 hr</td>
<td>2.14</td>
<td>869.5</td>
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<tr>
<td>37 30 min</td>
<td>1.64</td>
<td>880.6</td>
</tr>
<tr>
<td>37 30 min</td>
<td>2.02</td>
<td>1123</td>
</tr>
<tr>
<td>37 30 min</td>
<td>1.74</td>
<td>1102.6</td>
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</tbody>
</table>

Table 1. Yield and purity metrics for individual bloodspot samples.