BECKMAN COULTER Life Sciences

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

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

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.

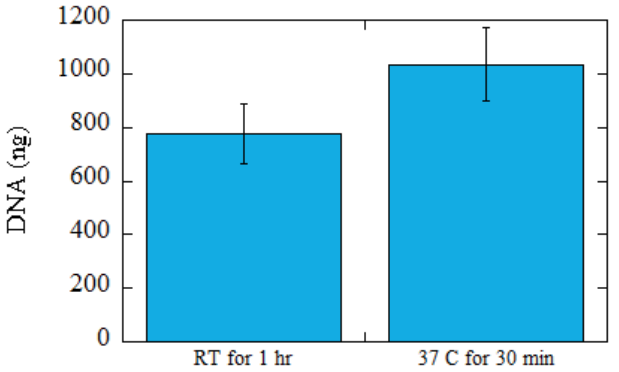

Figure 1. Yield of DNA from bloodspots with varying lysis conditions. Yield was measured by picogreen fluorescence and error bars represent the standard deviation of three technical replicates.

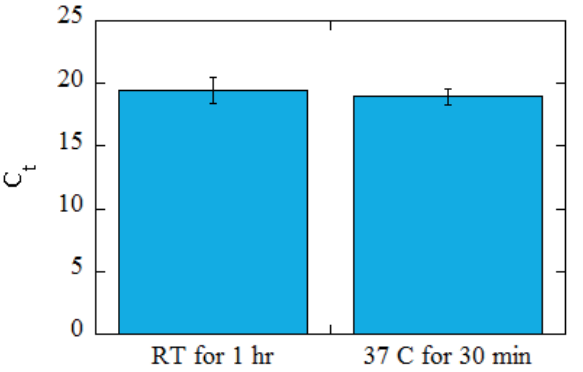

Figure 2. Data from qPCR of bloodspot DNA (100 ng) with ActB primers. Both lysis conditions remove PCR inhibitors in equal amounts. Error bars represent the standard deviation of three technical replicates.

SAMPLE ID	260/280	YIELD (NG, PICOGREEN)
RT 1 hr	2.01	803.3
RT 1 hr	1.89	651.4
RT 1 hr	2.14	869.5
37 30 min	1.64	880.6
37 30 min	2.02	1123
37 30 min	1.74	1102.6

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

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

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