EchoLUTION Blood DNA Kit – Protocol for dried blood spots (DBS)

for single-step purification of genomic DNA from dried blood spots

This kit contains a resealing valve integrated into the cap. The valves prevent any contamination from outside and from DNA preparations performed in parallel.

Materials and equipment needed.

- Use 1 to 5 punched-out circles (3 mm diameter) of dried blood spots per sample.
- Microcentrifuge with rotor for 1.5 mL and 2 mL reaction tubes.
  Important: Switch to relative centrifugal force, rcf (x g); if this is not possible, please use formula below* to calculate the conversion of rpm into rcf.
- Thermal shaker with agitation (for faster performance), capable of heating to 60 °C and 80 °C. Alternatively: heating block or heat chamber.
- Vortex mixer.
- Reaction tubes of 1.5 mL and 2 mL for lysis, column preparation, and elution.
- Pipets for 10 µL and 200 µL scale and corresponding pipet tips.
- For fastest procedure (PROTOCOL 1): **Cap Puncher** (BioEcho product no. 050-001-001).

Preparation before starting.

- Heat the thermal shaker, heating block or heat chamber to 60 °C.
- Set the microcentrifuge to **1,000 x g.**
  Important: Switch to relative centrifugal force, rcf (x g, not rpm);

BioEcho cap puncher

(scan QR code to watch a video).

PROTOCOL 1: Purification **using** the Cap Puncher

Lysis

1. For each sample, transfer **100 µL Blood Lysis Buffer** (LB) and **10 µL TurboLyse B Protease** (P) to a 1.5 mL reaction tube, preferably safe lock.
   If working with more than two samples, prepare a pre-mix with a final volume that is 10 % larger than required for the number of samples (see table). Transfer 100 µL to each tube.

Pre-mix calculation with examples.

No of samples	1	6 (+ 10 %)	12 (+ 10 %)	Yours
(P) TurboLyse B Protease (µL)	10	66	132	
(LB) Blood Lysis Buffer (µL)	100	660	1320	
Final volume (µL)	**110**	**762**	**1452**	

* Most centrifuges offer the choice between rpm and *g*-force (rcf); if not, calculate the rpm corresponding to 1,000 x *g* using the formula: rpm = 1,000 x $\sqrt{(\frac{1{,}000}{1.12 \, x \, r^2})}$, where r = radius of rotor in mm. E. g., with a radius r of 150 mm, the corresponding rpm to 1,000) x g is approx. 2,600 rpm.

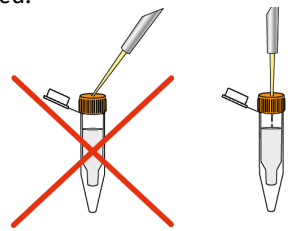
2. Add **1 to 5 punched-out circles (3 mm diameter)** of dried blood spots per sample. Vortex briefly.
3. Place the tube in the thermal shaker and incubate at **60 °C for 30 min** with agitation at full speed. Alternatively, incubate on a heating block for 60 min and pulse-vortex 3 times during lysis. Lysis time can be **shortened to 15 minutes** without loss in PCR performance but A_{260}/A_{230} purity ratio may be lowered.
   During lysis, proceed with step 5 column preparation (below).
4. After incubation at 60 °C, increase the temperature to **80 °C** and incubate for additional **10 min** with max. agitation.

Column preparation

5. Vortex the **EchoLUTION Spin Column** briefly and place into a **2 mL** reaction tube. Let stand for 10 – 20 min.
6. Use of the **Cap Puncher** (scan QR code to watch a video): Punch a hole into the column cap and lift the column together with the **Cap Puncher** out of the 2 mL collection tube. Snap off bottom closure of the column and detach the **Cap Puncher**.
   Place the punched spin column back into the 2 mL reaction tube.
7. Centrifuge **for 1 min** at **1,000 x g.** Discard the 2 mL reaction tube containing the column buffer.
8. Place the prepared **EchoLUTION Spin Column** into a new 1.5 mL reaction tube for elution of the sample DNA and place back into in the rack.
   Continue with purification of DNA.

Purification of DNA.

9. After having performed step 4, **add 10 µL Clearing Solution B** (CS) to each sample and vortex 3 sec. The sample will become cloudy.
   Note: Usually the addition of RNase is not required due to endogenous RNases. If RNA needs to be stringently degraded, add 1 µL RNase, DNase-free (BioEcho product no. 010-901-001) before the addition of **Clearing Solution B** and incubate for 2 min at room temperature.
10. Centrifuge for **2 min** at **maximum speed**.
11. Transfer supernatant containing the DNA onto the prepared column from step 8 as illustrated:



Insert pipet tip vertically through the hole in the column cap until forced through the lid valve (slight pressure release; otherwise, re-apply the pipet tip). Pipet the sample slowly (~5 sec) into the column.

12. Centrifuge **1 min** at **1,000 *x g***. The purified DNA flows through the column into the 1.5 mL elution tube. Discard the spin column.

The eluted DNA can be used immediately or stored at 4 °C or for long-term storage at - 20 °C. For spectrophotometric analysis, use **Low-TE Buffer** (T) supplied with the kit.

PROTOCOL 2: Purification **without** a Cap Puncher

Lysis

1. Perform steps 1-4 from PROTOCOL 1.

Column preparation

5. Vortex the **EchoLUTION Spin Column** briefly and place into a **2 mL** reaction tube. Let stand for 10 – 20 min.
5. **Loosen** the screw cap of the spin column **half a turn** and **snap off the bottom closure.**
   Important: Do not close the screw cap of the spin column. The screw cap must stay loosened **half a turn** to avoid generation of a vacuum. Place the column back into the 2 mL collection tube and both into the centrifuge.
6. Centrifuge for **1 min at 1,000 x g.** Discard the 2 mL reaction tube containing the column buffer. Do not reclose the column lid after centrifugation.
7. Place the prepared spin column into a new 1.5 mL reaction tube for elution of the sample DNA and place back into in the rack.
   Continue with purification of DNA.

Purification of DNA.

8. After having performed step 4, **add 10 µL Clearing Solution B** (CS) to each sample and vortex 3 sec. The sample will become cloudy.
   Note: Usually the addition of RNase is not required due to endogenous RNases. If RNA needs to be stringently degraded, add 1 µL RNase, DNase-free (BioEcho product no. 010-901-001) before the addition of **Clearing Solution B** and incubate for 2 min at room temperature.
9. Centrifuge for **2 min** at **maximum speed**.
10. Open the spin column and transfer supernatant containing the DNA onto the prepared column from step 8. Pipet slowly, drop-by-drop, and vertically onto the middle of the spin column to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading! Do not close the screw cap completely, screw cup must stay loosened half turn.
11. Centrifuge **1 min** at **1,000 *x g***. The purified DNA flows through the column into the 1.5 mL elution tube. Discard the spin column.

The eluted DNA can be used immediately or stored at 4 °C or for long-term storage at - 20 °C. For spectrophotometric analysis, use **Low-TE Buffer** (T) supplied with the kit.

Product use limitation

The EchoLUTION Blood DNA Kit is for research use only. It is not registered or authorized to be used for diagnosis, prevention, or treatment of a disease.

EchoLUTION Blood DNA Kit

for single-step purification of genomic DNA from dried blood spots

Product no.	010-001-010 (10)	010-001-050 (50)	010-001-250 (250)
Kit contents	Blood Lysis Buffer, TurboLyse B Protease, Clearing Solution B, Low-TE Buffer, and Spin Columns		

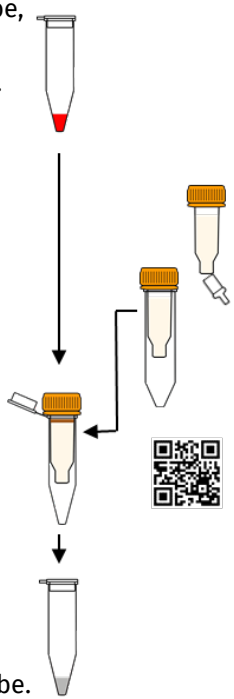
Quick Protocol (please, read extended protocol first)

Lysis and sample clearing

- Transfer **100 µL** (LB) and **10 µL** (P) to a 1.5 mL reaction tube, vortex briefly.
- Add **1 to 5 punched-out dried blood spots**, vortex briefly.
- Incubate **30 min at 60 °C** with maximum agitation.
- Incubate **10 min at 80 °C** with maximum agitation.

Column preparation (during 60 °C and 80 °C incubation)

- Homogenize column resin by vortexing and place in a 2 mL tube, let stand for 10 – 20 min.
- Punch a hole in the cap, and break off bottom closure (scan QR code to watch a video).
- Place spin column back into 2 mL tube.
- Centrifuge **1 min at 1,000 x g** to elute column buffer.
- Place column in a 1.5 mL tube for sample loading.



Purification of DNA

- Add **10 µL** (CS) and vortex shortly.
- Centrifuge. For **2 min at full speed.**
- Transfer lysate by pipetting slowly through cap hole – see PROTOCOL 1 or watch video (scan QR code).
- Centrifuge **1 min at 1,000 x g** to elute DNA into elution tube.
- Eluted DNA is ready to use.

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