EchoLUTION Blood DNA Kit – Protocol for dried blood spots

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

This protocol has been developed for 1 to 5 punched-out dried blood spots (~ 3 mm).

This kit contains a new column format with resealing valves 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 dried blood spots (~ 3 mm) per sample.
- Microcentrifuge with rotor for 1.5 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.
- For fastest performance: Thermomixer, capable of heating to 60°C with agitation; pre-heat to 60 °C. Alternatively: Heating Block, pre-heated to 60°C
- Vortexer
- One reaction tube (1.5 ml) per sample for the lysis step (preferably safe-lock).
- One reaction tube (2 ml) per sample for column preparation.
- One reaction tube (1.5 ml) per sample for elution and collection of the purified DNA.
- Pipets for 10 µl and 200 µl scale, corresponding pipet tips.
- For fastest procedure (PROTOCOL 1): Cap Puncher (BioEcho product no. 050-001-001).

Preparation before starting

- Heat the thermomixer or thermo block to 60°C.
- Set the microcentrifuge to **1000 x *g*.***
  Important: switch to centrifugal force, 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 60 µl to each tube.

   Table: Pre-mix calculation with examples (see cap labels).

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**	

2. Add **1 to 5 punched-out dried blood spots (~ 3 mm)**. Vortex briefly.
3. Place the tube in the thermomixer 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.

   Meanwhile during lysis, **proceed with step 5** of "Column Preparation" (below).
4. Increase the temperature to **80°C** and incubate for additional **10 min.**

Column preparation

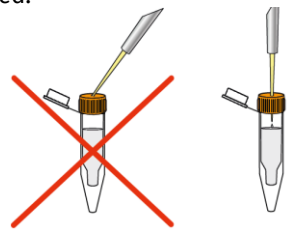
5. Vortex the EchoLUTION Spin Column briefly and place into a 2 ml reaction tube. Let stand for 10-20 min.
6. Use of the cap puncher (scan QR code to watch a video): Punch a hole into the column cap and lift the column together with the Cap Puncher out of the 2 ml collection tube. Snap off bottom closure 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 1000 x *g**.** Discard the 2 ml reaction tube containing the column buffer.
8. 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" (below).

Purification

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 A before the addition of Clearing Solution 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 1000 *x g****. The purified DNA (50–80 µl, blood donor-specific) 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 1x Tris Buffer (T) supplied with the kit.

PROTOCOL 2: Purification without a Cap Puncher

Lysis

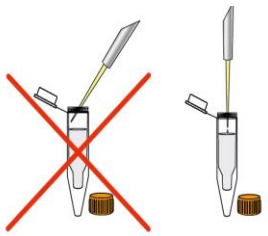
1. Perform steps 1-4 from PROTOCOL 1.

Column preparation

5. Vortex the EchoLUTION Spin Column briefly and place into a 2 ml reaction tube. Let stand for about 10-20 min (recommended during lysis step).
6. **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.
7. Centrifuge for **1 min at 1000 x *g**.** Discard the 2 ml reaction tube containing the column buffer. Do not reclose the column lid after centrifugation.
8. 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" (below).

Purification

9. After having performed step 4, **add 10 µl Clearing Solution B** (CS) to each sample. Vortex 3 sec to mix. 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 A before the addition of Clearing Solution 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:



Open cap and pipet the sample slowly (~5 sec) onto the center of the resin bed of the prepared spin column. Close screw cap and loosen again half a turn.
Important: Do not close the screw cap of the spin column tightly!

12. Centrifuge **1 min at 1000 *x g**.** The purified genomic DNA (50–80 µl, blood donor-specific) flows through the column into the 1.5 ml elution tube. Discard the spin column.

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.

* Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm corresponding to 1000 x g using the formula: rpm = 1,000 x $\sqrt{(\frac{1000}{1.12 \times r})}$, where r = radius of rotor in mm. E. g., with a radius r of 150 mm, the corresponding rpm to 1000) x g is approx. 2,600 rpm.

EchoLUTION Blood DNA Kit

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

Product no. (rxn's)	010-001-010 (10)	010-001-050 (50)	010-001-250 (250)
Kit contents	Blood Lysis Buffer, TurboLyse B Protease, Clearing Solution B, 1x Tris Buffer, Spin Columns		

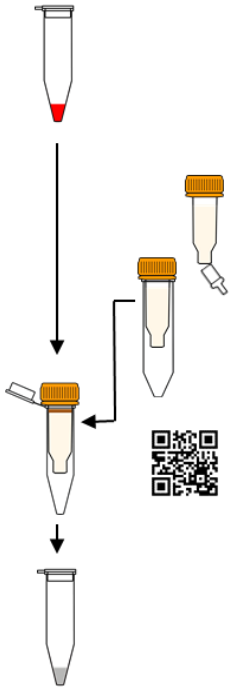
Quick PROTOCOL using the Cap Puncher

Lysis and sample clearing

- Transfer **100 µl** (LB) **+ 10 µl** (P) to 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 5 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 1000 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 1000 *x g**.**

Purified DNA is ready to use

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BioEcho Life Sciences GmbH
Nattermannallee 1
50829 Köln (Cologne)/Germany
Phone: +49 (0) 221-99 88 97-0
E-Mail: contact@bioecho.de
www.bioecho.de