EchoLUTION Blood DNA Micro Kit – Protocols

for single-step purification of genomic DNA from liquid blood samples

This protocol has been developed for up to 60 µl (fresh, stabilized or frozen) of human or animal whole blood (EDTA-, Citrate- or Heparin-stabilized) or buffy coats.

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

- **60 µl liquid blood** per sample
- **Microcentrifuge** with rotor for 1.5 and 2 ml reaction tubes
  Important: Switch centrifuge to *relative centrifugal force, rcf* (x *g**); if this is not possible please use formula below* to calculate the conversion of round per minute (rpm) into rcf.
- **Thermal shaker** with **agitation** (for fastest performance), capable of heating to 60°C and 80°C. Alternatively: Heating Block or heat chamber
- **Vortexer**
- **Pipets** for 10 µl and 200 µl scales, corresponding pipet tips
- 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
- For fastest procedure (PROTOCOL 1): Cap Puncher (BioEcho product no. 050-001-001)

Preparation before starting

- Heat the thermal shaker or thermo block 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

PROTOCOL 1: Purification using the Cap Puncher

The spin column caps contain resealing valves that prevent any sample contamination through the cap after it has been punched. During sample loading, make sure to pierce the valve with the pipet tip; this is indicated by a slight pressure release.

Lysis

1. For each sample, transfer **50 µl Blood Lysis Buffer** (LB) and **10 µl TurboLyse B Protease Mix** (P) to a 1.5 ml reaction tube (preferably safe-lock) and add up to 60 µl blood. If working with more than two samples, prepare a **Lysis Master Mix** with 10% excess volume for the number of blood samples (see table).

   Lysis Master Mix:

No of samples	1	6 (+10%)	12 (+10 %)	Yours
(LB) Blood Lysis Buffer (µl)	50	330	660	
(P) TurboLyse B Protease (µl)	10	70	130	
Final volume (µl)	**60**	**400**	**790**	

   Add **60 µl of the Lysis Master Mix** to each **60µl blood sample**. Vortex briefly.

2. Place the reaction tube(s) in the thermal shaker and incubate at **60°C** for **30 min** with max. agitation. 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"

3. After incubation at 60°C, increase the temperature to **80°C** and incubate for additional **10 min** with max. agitation.

Column preparation (during steps 2 and 3)

4. Vortex the **EchoLUTION Spin Column** briefly and place into a **2 ml** reaction tube. Let stand for 10-20 min.
5. 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 by twisting clockwise while pulling out. Place the punched spin column back into the 2 ml reaction tube.
6. Centrifuge for **1 min** at **1,000 x *g****. Discard the flow-through volume ("void volume") collected in the 2 ml reaction tube.
7. Place the prepared **EchoLUTION Spin Column** into a new **1.5 ml** reaction tube for elution of the purified DNA and place back into the rack.

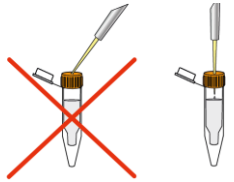
   Continue with "Purification of DNA".

Purification of DNA

8. After having performed step 3, 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** before the addition of Clearing Solution B and incubate for **2 min** at room temperature.

   For extraction from buffy coat, pipet the lysate **up and down 10 times** before proceeding to centrifugation (step 10).
9. Centrifuge for **2 min** at **max. speed**.
10. Transfer **lysis supernatant (max. 100 µl)** containing the DNA onto the prepared **EchoLUTION Spin Column** from step 7 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.

    Note:
    - Residual sample particles may be loaded and will not interfere with purification.
11. Centrifuge for **1 min** at **1,000 *x g****. The purified DNA (50–80 µl, 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 kept at 2-8°C or for long-term storage at –20°C. For spectrophotometric analysis, use the **1x Tris Buffer** (T) supplied with the kit.

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

Lysis

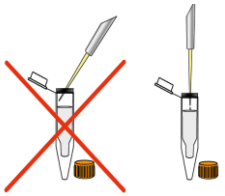
1. Perform steps 1-3 from PROTOCOL 1.

Column preparation

4. Vortex the **EchoLUTION Spin Column** briefly and place into a **2 ml** reaction tube. Let stand for 10-20 min (recommended to be done during lysis step).
5. **Loosen** the screw cap of the spin column **half a turn** and **snap off the bottom closure**.
   Important: Do not re-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.
7. Place the prepared spin column into a new **1.5 ml** reaction tube for elution of the sample DNA and place back into the rack.
   Continue with "Purification of DNA".

Purification of DNA

8. After having performed step 3, 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** before the addition of Clearing Solution B and incubate for **2 min** at room temperature
9. Centrifuge for **2 min** at **max. speed**.
10. Transfer **lysate supernatant (max. 100 µl)** containing the DNA onto the prepared column from step 7 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 re-close the screw cap of the spin column completely!

Note:

- During loading of lysate, do not touch the resin bed with your pipette tip!
- Residual sample particles may be loaded and will not interfere with purification.

11. Centrifuge **1 min** at **1,000 x *g**. The purified DNA elutes into the 1.5 ml elution tube and can be immediately applied in downstream applications.

Product use limitation

The EchoLUTION Blood DNA Micro 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 matching the g-force using the formula: rpm = 1,000 x $\sqrt{(\frac{g}{1.12\,x\,r})}$, where r = radius of rotor in mm. and g the required g-force.
E. g., with a radius of 150 mm, the corresponding rpm to 1,000 x g is approx. 2,400 rpm.

EchoLUTION Blood DNA Micro Kit

for single-step purification of genomic DNA from liquid blood samples

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

Quick PROTOCOL (please read protocol first)

Lysis

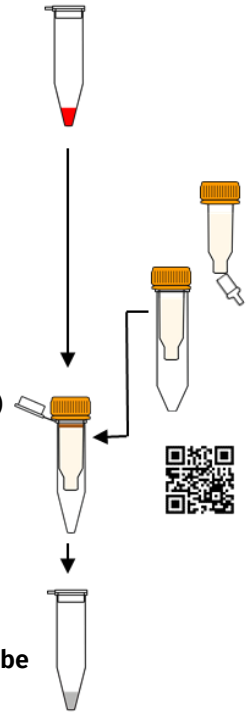
- Transfer **50 µl** (LB) + **10 µl** (P) to reaction tube
- Add **60 µl blood**, vortex briefly
- Incubate **30 min** at **60°C**, max. agitation
- Incubate **10 min** at **80°C**, max. agitation
- Add **10 µl** (CS) and vortex shortly
- Centrifuge 2 **min** at **max. speed**

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

- Vortex **EchoLUTION spin column** and place in a **2 ml** tube Let stand for 10 min
- Punch a hole in the cap with the Cap Puncher, 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

Purification of DNA

- Transfer **lysate supernatant (max. 100 µl)** by pipetting **slowly** through cap hole (scan QR code to watch a video)
- Centrifuge **1 min** at **1,000 x *g**** to elute DNA into **Elution tube**
- Eluted DNA is ready to use



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