EchoLUTION Blood DNA Kit – Protocols

for 1-step purification of genomic DNA from liquid blood

This protocol has been developed for isolation of gDNA from up to 60 µl of human or animal whole blood (fresh or stabilized, e. g., EDTA, Citrate or Heparin), serum or plasma.

Materials and equipment needed

- Use 60 µl blood (fresh, treated with EDTA, citrate or heparin) per sample.
- Microcentrifuge with rotor for 1.5 and 2 ml reaction tubes*
- For fastest performance: Thermomixer, capable of heating to 60°C and 80°C with agitation (full speed at 800–1500 rpm). Pre-heated 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 genomic DNA
- Pipets for 10 µl and up to 200 µl, corresponding pipet tips
- For fastest procedure (PROTOCOL1): Cap Puncher (BioEcho product no. 050-001-001)

Preparation before starting

- Heat the thermomixer or thermo block to 60°C.
- Set the microcentrifuge to 750 x g.*

PROTOCOL 1: Purification using the Cap Puncher

Lysis

1. **For each sample, transfer 50 µl Blood Lysis Buffer** (BL) **and 10 µl TurboLyse B&C Protease Mix** (TLP) **to a 1.5 ml reaction tube,** preferably safe-lock. **Mix by flicking or vortexing.**
   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 lids.

No of samples	**1**	**6** (+10%)	**12** (+10 %)	**Yours**
(TLP) TurboLyse B&C Protease Mix (µl)	10	66	132	
(BL) Blood Lysis Buffer (µl)	50	330	660	
Final volume (µl)	**60**	**396**	**792**	

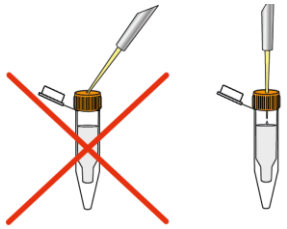
2. **Add 60 µl blood.** Do not use more than 60 µl blood. **Mix by vortexing briefly.**
3. **Place the tube in the thermomixer and incubate at 60°C for 15 minutes with agitation at full speed.** Alternatively, incubate on a heating block for 60 min and pulse-vortex 3 times during lysis. **During incubation**, vortex the suspension in the EchoLUTION Spin Column **as described in step 5**.
4. **Increase the temperature to 80°C and incubate for additional 10 minutes. Meanwhile, proceed with step 6** of "Column Preparation" (below).

Column preparation

5. **Vortex the EchoLUTION Spin Column briefly** and place into a 2 ml reaction tube. **Let stand for 15-20 min.**
6. **Use the cap puncher** (scan QR code to watch a video)**:** Place the 2 ml tube with the spin column into a rack. Punch a hole into the cap and lift the column together with the Cap Puncher out of the collection tube. Snap off bottom closure of the column and detach the Cap Puncher. Place back the spin column into the 2 ml reaction tube.
7. **Centrifuge for 1 minute at 750 x *g**.** Discard the 2 ml reaction tube containing the flow-through.
8. **Place the prepared spin column into a new 1.5 ml reaction tube** for elution of the sample and both together in a rack. Continue with "Purification" (below).

Purification

9. **After having performed step 4, add 20 µl Clearing Solution** (CS) **to each sample. Vortex 3 sec to mix.** The sample will become cloudy. **Important: Do not centrifuge!** Optional: Add 1 µl RNase A and let stand for 2 min at room temperature to exclude any RNA traces.
10. **Transfer 90 – 110 µl lysate** containing the DNA to the prepared column from step 8 **as illustrated:**



Completely insert pipet tip vertically through the hole in the column cap and pipet the lysed sample slowly (~5 sec) into the column.

11. **Centrifuge 1 minute at 750 *x g*.** The purified genomic DNA (90–100 µl; 10 mM Tris·Cl, pH 7.8) flows through the column into the 1.5 ml elution tube. Discard the spin column.
12. **To ensure long-term DNA stability,** add 1/100 volume (0.9 µl for 90 µl eluate) Protector Solution (PS) to the purified DNA and mix.
13. **The eluted genomic DNA can be used immediately or stored at 4°C or –20°C. Before spectrophotometric analysis,** be sure that the concentration of salt in sample and blank are the same. Differences may influence the A_{260}/A_{230} purity ratio.

PROTOCOL 2: Purification without a Cap Puncher

Lysis

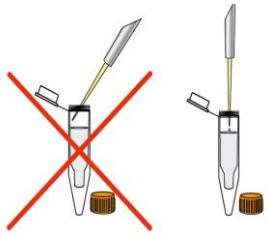
1. **Perform steps 1-4 from PROTOCOL 1.**

Column preparation

5. **Vortex the EchoLUTION Spin Column briefly** and place into a 2 ml reaction tube. **Let stand for 15 - 20 min.**
6. **Loosen the screw cap of the spin column half a turn and snap off the bottom closure.** **Important: Don´t close the screw cap of the spin column.** The screw cap must stay loosened half a turn to avoid generation of a vacuum. Place back the column into the 2 ml collection tube.
7. **Centrifuge for 1 minute at 750 x *g*.** See section "Materials and equipment needed" above for details. Discard the 2 ml reaction tube containing the flow-through.
8. **Place the prepared spin column into a new 1.5 ml reaction tube for elution** of the sample and both together in a rack. **Continue with "Purification" (below).**

Purification

9. **After having performed step 4, add 20 µl Clearing Solution (CS) to each sample. Vortex 3 sec to mix.** The sample will become cloudy. **Important: Do not centrifuge!** Optional: Add 1 µl RNase A (R) and let stand for 2 min at room temperature to exclude any RNA traces.
10. **Transfer 90 – 110 µl lysate containing the DNA to the prepared column from step 8 as illustrated:**



Open cap and pipet the lysed 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: Don´t close the screw cap of the spin column tightly!**

11. **Perform steps 11 – 13 of Protocol 1 using the Cap Puncher.**

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.

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* Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm corresponding to 750 x g using the formula: rpm = 1000 x $\sqrt{(\frac{750}{1.12 \times r})}$, where r = radius of rotor in mm. E.g., with a radius of 150 mm, the corresponding rpm to 750 x g is approx. 2200 rpm.

EchoLUTION Blood DNA Kit

for 1-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, RNAse A, Clearing Solution, Protector Solution, Tris Buffer, Spin Columns		

Quick PROTOCOL 1

Lysis and sample clearing

- Transfer 60 µl (BL) + 10 µl (TLP) to reaction tube, vortex briefly.
- Add 60 µl blood, vortex briefly.
- Incubate 15 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)

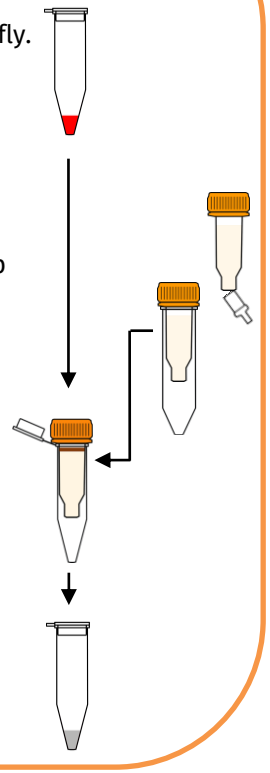
- Homogenize by vortexing. Let stand for 15-20 min
  Place in a 2 ml tube, punch a hole in the cap using the Cap Puncher, and break off bottom closure.
- Centrifuge 1 min at 750 *x g*.
- Place column in a 1.5 ml tube for elution.

Purification of lysate

- Add 20 µl (CS) and vortex. Do not centrifuge.
  Transfer 90-110 µl lysate by pipetting slowly through cap hole – see PROTOCOL 1 or watch video (scan QR code)
- Centrifuge 1 min at 750 *x g*.

Purified genomic DNA

- 90–100 µl in Tris·Cl Buffer pH 7.8
- Add 1/100 volume (0.9 µl) (PS).



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