EchoLUTION Blood DNA HiYield Kit – Protocols

for single-step purification of genomic DNA from liquid blood samples ≥ 200µl

This protocol has been developed for gDNA extraction from 200 µl to 1,000 µl (fresh, stabilized or frozen) of human or animal whole blood (EDTA-, Citrate- or Heparin-stabilized). 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 200 µl to 1,000 µl blood 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 rcf into rpm.
- 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 or 2.0 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, 200 µl and 1,000 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 **2,000 x *g* *.**
  Important: switch to *relative centrifugal force*, not rpm


BioEcho Cap Puncher

PROTOCOL 1: Purification using the Cap Puncher

Lysis

1. Vortex blood tube and transfer each blood sample into a reaction tube (preferably safe-lock) and add **Erythrocyte Lysis Buffer HY** (ELB) according to the following table:

Blood sample volume	200 – 500 µl	> 500 µl
to reaction vessel	1.5 ml tube	2.0 ml tube
Add (ELB) ad **	1.3 ml final volume	2.0 ml final volume

** e.g. if starting with 300 µl blood, add 1,000 µl ELB

Mix by vortexing and incubate for 3 min at room temperature for erythrocyte lysis. Completion of lysis is indicated by a decrease in turbidity.

2. Spin down white blood cells for **1 min at 2,000 x g*** and completely remove supernatant. Repeat steps 1 and 2 once (resuspend cell pellet using (ELB)) if:
   - blood sample volume > 500 µl
   - white blood cell pellet is not clearly visible (e. g., with older blood samples)
3. Resuspend **Blood Lysis Buffer HY** (LB) by 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).

Table: Pre-mix calculation with examples:

No of samples	1	6 (+10%)	12 (+10 %)	Yours
(P) TurboLyse HY Protease Mix (µl)	25	165	330	
(LB) Blood Lysis Buffer HY (µl)	55	363	726	
Final volume (µl)	**80**	**528**	**1056**	

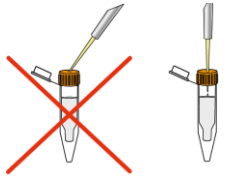
4. Add **25 µl** (P) **and 55 µl** (LB) **(80 µl Pre-mix)** to the pellet and resuspend by pulse vortexing. Incubate with shaking for 5 min at 60°C. Alternatively, incubate on a heating block for 10 min with pulse-vortexing twice during incubation.
   During lysis, **proceed with step 5** of "Column Preparation" (below).

Column preparation

5. Vortex the EchoLUTION Spin Column briefly and place into a 2 ml reaction tube. Let stand for about 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. Do not reclose the column lid after centrifugation.6511
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 HY** (CS) to each sample and pulse vortex 10 sec. The sample will become cloudy.
   Note: The addition of RNase is not required due to endogenous RNases.
10. **Homogenize the sample by vigorously pipetting up and down for at least 10 times** using a 200 µl tip. Make sure to resuspend particulates completely and to reduce viscosity when processing ≥ 500 µl blood.
11. Centrifuge for **2 min at maximum speed.**
12. Transfer supernatant (80 to max. 110 µl) 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.

13. Centrifuge **1 min at 1,000 *x g**.** The purified DNA (about 95 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) as blank supplied with the kit.

PROTOCOL 2: Purification without a Cap Puncher

Lysis

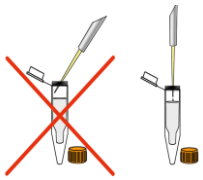
1. Perform steps 1-4 from PROTOCOL 1.

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 1,000 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 HY** (CS) to each sample. Vortex 10 sec to mix. The sample will become cloudy.
   Note: The addition of RNase is not required due to endogenous RNases.
10. Homogenize the sample by at least 10 times pipetting up and down.
11. Centrifuge for **2 min at maximum speed.**
12. Transfer supernatant (80 to max. 110 µl) 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!

13. Centrifuge **1 min at 1,000 *x g**.** The purified genomic DNA (about 95 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 HiYield 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. 3,300 rpm.

EchoLUTION Blood DNA HiYield Kit

for single-step purification of genomic DNA from liquid blood samples ≥ 200µl

Product no. (rxn's)	010-011-010 (10)	010-011-050 (50)	010-011-250 (2x125)
Kit contents	Erythrocyte Lysis Buffer, Blood Lysis Buffer HY, Clearing Solution HY, TurboLyse HY Protease, 1x Tris Buffer, EchoLUTION Spin Columns		

Quick PROTOCOL 1

Lysis and sample clearing

- Add blood sample to reaction vessel and add (ELB) as described
- Mix by vortexing and incubate for **3 min** at room temperature
- Spin down white blood cells for **1 min at 2,000 x *g**** and completely remove supernatant
- Transfer **55 µl** (LB) **+ 25 µl** (P) to the white blood cell pellet, mix by pulse vortexing
- Incubate **5 min at 60°C** with maximum agitation

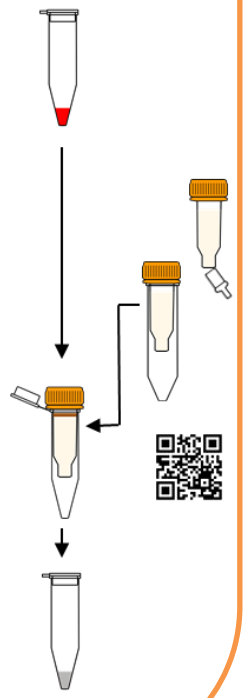
Column preparation (during 60°C incubation)

- Homogenize column resin by vortexing and place in a 2 ml tube, let stand for about 10 min
- Punch a hole in the cap, **open the column ½ turn** 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
- Homogenize sample by pipetting **up and down 10 times**
- Centrifuge **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****

Purified DNA is ready to use.



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