EchoLUTION Tissue DNA Micro Kit – Protocol

for single-step purification of genomic DNA from tissue samples

This protocol has been developed for fresh, frozen and stabilized human and animal tissue samples including DNase-rich (e. g., spleen, liver, kidney) and lipid tissues (e.g. brain, fat).

Materials and equipment needed

- 1 to 20 mg tissue per sample depending on tissue type.
  Sample input recommendation:

Tissue type	Generic	High DNA content (e g., spleen, liver, kidney)	Low DNA content (e. g., muscle, cartilage)
Amount	10 mg	5 mg	20 mg

- 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 rounds 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.
- Ceramic Blade Scalpels. BioEcho product no. 050-002-001
- 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)

PROTOCOL 1: Purification using the Cap Puncher

BioEcho Cap Puncher

Lysis

1. Transfer tissue sample to the bottom of a 1.5 ml reaction tube (preferably safe-lock) while the tube is cooled on ice (or cooling block) to avoid DNA degradation during sample loading.
   Note:
   - If possible, cut tissue into small pieces to speed up lysis.
   - For stabilized tissue samples briefly rinse with water to remove traces of stabilization solution before adding samples to the reaction tube.
2. Add 90 µl Tissue Lysis Buffer (P) and 5 µl TurboLyse T Protease (LB) to each tissue sample. Mix by flicking or vortexing.
   If working with more than two samples, prepare a Lysis Master Mix with 10% excess volume for the number of tissue samples (see table).

   Lysis Master Mix:

No of samples	1	6 (+10%)	12 (+10 %)	Yours
(P) Tissue Lysis Buffer (µl)	90	600	1,190	
(LB) TurboLyse T Protease (µl)	5	35	70	
Final volume (µl)	95	635	1,260	

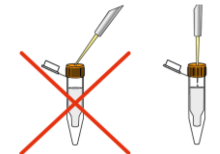
   Add 95 µl of the Lysis Master Mix to each tissue sample.
3. Place the reaction tube(s) in the thermal shaker and incubate at 60°C for 30 min with max. agitation (for 60 min if agitation is not feasible, in this case pulse-vortex 3 times during lysis).
   Note: If samples are not completely lysed after the time period described above, continue with the next step. Residual cellular debris will not interfere with the purification performance.
   Note: For some tissue types, lysis is already complete after 15 min. Step 3 may be shortened accordingly.
   Meanwhile during lysis, proceed with step 5, "Column preparation".
4. After incubation at 60°C, increase the temperature to 80°C and incubate for additional 10 min with max. agitation.

Column preparation (during step 3. and 4.)

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 by twisting while pulling out. Place the punched spin column back into the 2 ml reaction tube.
7. Centrifuge for 1 min at 1,000 x *g**. Discard the flow-through volume ("void volume") collected in the 2 ml reaction tube.
8. 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

9. **Optional**: After having performed step 4, add 1 µl RNase (R) to each lysed sample and vortex 3 sec. Incubate for 2 min at room temperature to remove RNA traces.
10. After having performed step 4, add 10 µl Clearing Solution T (CS) to each sample. Vortex 3 sec. The sample becomes cloudy.
11. Centrifuge for 2 min at full speed.
12. Transfer lysis supernatant (max. 100 µl) containing the DNA onto the prepared EchoLUTION Spin Column from step 8 as illustrated:
    Insert pipet tip vertically through the hole in the column cap and pipet the sample slowly (~5 sec) into the column.



    Note:
    - During loading of lysate, do not touch the resin bed!
    - Residual sample particles may be loaded and will not interfere with purification.
13. Centrifuge for 1 min at 1000 x *g**. The purified DNA elutes into the 1.5 ml elution tube and can be immediately applied in downstream applications.

EchoLUTION Tissue DNA Micro Kit

for single-step purification of genomic DNA from tissue samples

Product no. (rxn's)	010-002-010 (10)	010-002-050 (50)	010-002-250 (250)
Kit contents	Tissue Lysis Buffer, TurboLyse T Protease, RNAse A Tissue, Clearing Solution T, 1x Tris Buffer, EchoLUTION Spin Columns		
Related products	Cap Puncher 050-001-001	Ceramic Blade Scalpels 050-002-00x	

Quick PROTOCOL (please read protocol first)

Lysis

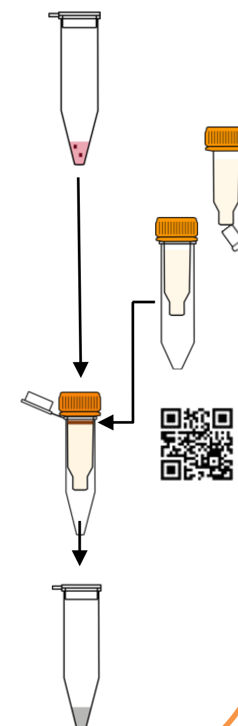
- Transfer 10 mg tissue to reaction tube
- Add 90 µl (LB)
- Add 5 µl (P), vortex briefly
- Incubate 30 min/60°C, then 10 min/80°C, max. agitation
- Add 1 µl (R), vortex shortly and incubate 2 min at room temperature
- Add 10 µl (CS) and vortex shortly
- Centrifuge 2 min at max. speed

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



PROTOCOL 2: Purification without a Cap Puncher

Lysis

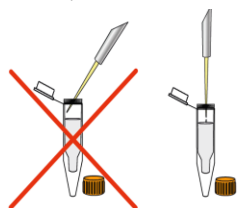
1. Perform steps 1-4 from PROTOCOL 1.

Column preparation

5. Vortex the EchoLUTION Spin Column briefly and place into a 2 ml reaction tube. Let stand for 10-20 min (recommended to be done during lysis step).
6. 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.
7. Centrifuge for 1 min at 1,000 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 the rack.
   Continue with "Purification of DNA".

Purification of DNA

9. After having performed step 4, add 1 µl RNase (R) to each lysed sample and vortex for 3 sec. Let stand for 2 min at room temperature to remove RNA traces.
10. Add 10 µl Clearing Solution T (CS) to each sample.
    Vortex 3 sec. The sample will become cloudy.
11. Centrifuge for 2 min at maximum speed.
12. Transfer lysate supernatant (max. 100 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 re-close the screw cap of the spin column completely!

Note:

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

13. 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 Tissue 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.

2020/01/23

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