EchoLUTION Tissue DNA Micro Kit – Protocols

for 1-step purification of genomic DNA from small tissue samples

This protocol has been developed for particularly low sample input and is suited for any tissue sample including DNase-rich tissues (e. g., liver, kidney and intestine).

Materials and equipment needed

- 0.1 to 10 mg fresh, frozen or stabilized tissue sample per prep (spleen: max. 5 mg)
- 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 (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 800 x g.*

PROTOCOL 1: Purification using the Cap Puncher

Lysis

1. **For each sample, transfer 80 µl Tissue Lysis Buffer** (TL) **and 3 µl TurboLyse T Pro-tease 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).

   Table: Pre-mix calculation with examples, see cap lids

No of samples	1	6 (+10%)	12 (+10 %)	Yours
TurboLyse T Protease (µl)	3	20	40	
(TL) Tissue Lysis Buffer (µl)	80	528	1056	
Final volume (µl)	**83**	**548**	**1096**	

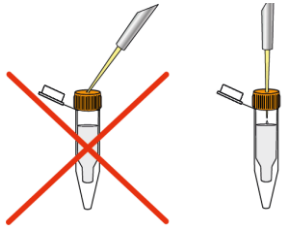
2. **Cut 0.1 to 10 mg tissue** (only for spleen: max. 5 mg) **into small pieces and place in a 1.5 ml reaction tube.** Keep fresh or frozen samples cold until all samples are prepared. **Note for stabilized tissue samples only**: briefly rinse with water to remove traces of stabilization solution before adding samples to the sample tube.
3. **Add 83 µl of the pre-mix from step 1 to each sample.**
4. **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. **During incubation**, prepare the EchoLUTION Spin Column **as described in steps 7-10**.
5. 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. If no remaining tissue is visible, the overall time can be shortened accordingly.
6. **Increase the temperature to 80°C and incubate for additional 10 min.** Meanwhile, continue with "Column Preparation" (below).

Column preparation

7. **Vortex the EchoLUTION Spin Column briefly** and place into a 2 ml reaction tube. **Let stand for 15-20 min.**
8. **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.
9. **Centrifuge for 1 min at 800 x *g**.** Discard the 2 ml reaction tube containing the flow-through.
10. **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 of lysate

11. **After having performed step 6, add 1 µl RNase A** (R) **to each lysed sample and vortex. Let stand for 2 min** at room temperature to remove RNA traces.
12. **Add 20 µl Clearing Solution** (CS) **to each sample. Vortex 3 sec to mix.** The sample becomes cloudy.
13. **Centrifuge for 3 min at maximum speed.**
14. **Transfer 90 – 110 µl supernatant** containing the DNA to the prepared column from step 10 **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.

15. **Centrifuge 1 min at 800 *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.
16. **To ensure long-term DNA stability,** add 1/100 volume (0.9 µl for 90 µl eluate) Protector Solution (P) to the purified DNA and mix.
17. **The eluted genomic DNA can be used immediately or stored at 4°C or –20°C. Before spectrophotometric analysis,** be sure that the concentrations 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

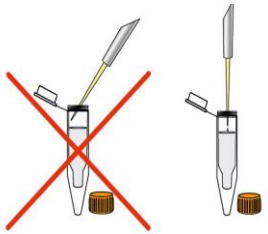
1. **Perform steps 1-6 from PROTOCOL 1.**

Column preparation

7. **Vortex the EchoLUTION Spin Column briefly** and place into a 2 ml reaction tube. **Let stand for 15-20 min.**
8. **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 the column back into the 2 ml collection tube.
9. **Centrifuge for 1 min at 800 x *g**.** Discard the 2 ml reaction tube containing the flow-through.
10. **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 of lysate

11. Upon completion of step 6 (PROTOCOL 1), add 1 µl RNase A (R) to each lysed sample and vortex. Let stand for 2 min at room temperature to remove RNA traces.
12. **Add 20 µl Clearing Solution** (CS) to each sample. **Vortex 3 sec** to mix.
13. **Centrifuge for 3 min at maximum speed.**
14. **Transfer 90 – 110 µl supernatant** containing the DNA to the prepared column from step 10 **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!**

15. **Perform steps 15 – 17 from PROTOCOL 1.**

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 corresponding to 800 x g using the formula: rpm = 1000 x $\sqrt{(\frac{800}{1.12\ x\ r})}$, where r = radius of rotor in mm. E.g., with a radius of 150 mm, the corresponding rpm to 800 x g is approx. 2200 rpm.

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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, Clearing Solution, Protector Solution, Tris Buffer, Spin Columns		

Quick PROTOCOL 1

Lysis

- Transfer 80 µl (TL) + 3 µl (TLP) to reaction tube, vortex briefly.
- Add 0.1 – 10 mg tissue, 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)

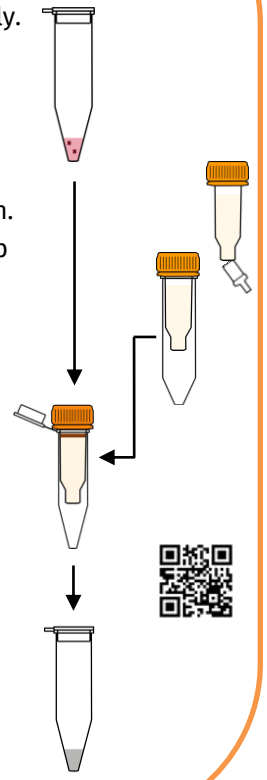
- Homogenize suspension by vortexing. Let stand for 20 min.
- Place in a 2 ml tube, punch a hole in the cap using the Cap Puncher, and break off bottom closure.
- Centrifuge 1 min at 800 *x g*.
- Place column in a 1.5 ml tube for elution.

Purification of lysate

- Add 1 µl (R), vortex, incubate 2 min at room temperature
- Add 20 µl (CS), vortex.
- Centrifuge 3 min at max. speed.
- Transfer 90-110 µl lysate by pipetting slowly through cap hole – see PROTOCOL 1 or watch video (scan QR code)
- Centrifuge 1 min at 800 *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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