EchoLUTION CellCulture DNA Kit – Protocols

for 1-step purification of genomic DNA from cell lines and primary cells

This protocol has been developed for gDNA isolation from any animal cell line and from primary cells including low sample input.

Materials and equipment needed

- Use up to 2x10^6 cells per prep. When using fresh cell suspensions, wash cells with phosphate-buffered saline (PBS) and pellet up to 2x10^6 cells in a 1.5 ml reaction tube for 5 minutes at 300 x g and carefully remove the supernatant. Keep cells at 0–6°C.
- 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. **Thaw frozen cell pellets and keep at 0–6°C. Add 60 µl cold PBS (not supplied) to cell pellet and resuspend by flicking or vortexing until homogenized.**
   For each sample, transfer 10 µl TurboLyse B&C Protease Mix (TLP) and 0.5 µl Cell Lysis Buffer (CL) 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
(TLP) TurboLyse B&C Protease Mix (µl)	10	66	132	
(CL) Cell Lysis Buffer (µl)	0.5	3.3	6.6	
Final volume (µl)	**10.5**	**69.3**	**138.6**	

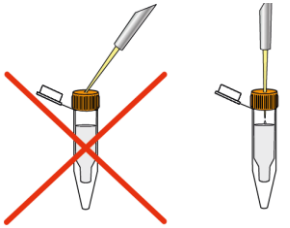
2. **Add 10.5 µl of the pre-mix from step 1 to each sample.**
3. **Place in the thermomixer immediately and incubate at 60°C for no longer than 2–3 minutes with agitation at full speed.** Alternatively incubate on a heating and pulse-vortex 3 times during lysis.
4. **Place samples at room temperature. Add 6 µl Cell Lysis Buffer and 2 µl RNase A (R) and vortex briefly.** 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 and add 8 µl per sample.
5. Further incubate samples in the thermomixer **at 60°C for 10 minutes** with agitation at full speed. Alternatively incubate on a heating block with 3 x pulse vortexing. **During incubation**, vortex the suspension in the EchoLUTION Spin Column **as described in step 7**.
6. **Increase the temperature to 80°C and incubate for additional 10 minutes. Meanwhile, proceed with step 8** of "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 minute at 750 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

11. **After having performed step 6, add 20 µl Clearing Solution (CS) to each sample. Vortex 3 sec to mix.** The sample becomes cloudy.
12. **Centrifuge for 3 minutes at maximum speed.**
13. **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.

14. **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.
15. **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.
16. **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 and clearing

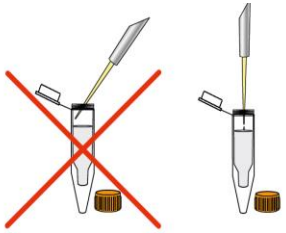
1. **Perform steps 1-6 from PROTOCOL 1.**

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 back the column into the 2 ml collection tube.
9. **Centrifuge for 1 minute at 750 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 from PROTOCOL 1, add 20 µl Clearing Solution (CS) to each sample. Vortex 3 sec to mix.
12. **Centrifuge for 3 minutes at maximum speed.**
13. **Transfer 90 – 110 µl supernatant** containing the DNA to the prepared column from step 6 **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!**

14. **Perform steps 14 – 16 of PROTOCOL 1.**

Product use limitation

The EchoLUTION CellCulture DNA 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 $\sqrt{(\frac{750}{1.12 \times r})}$, 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.

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Product no. (rxn's)	**010-006-010** (10)	**010-006-050** (50)	**010-006-250** (250)
Kit contents	Cell Lysis Buffer, TurboLyse B&C Protease, RNAse A, Clearing Solution, Protector Solution, Tris Buffer, Spin Columns		

Quick PROTOCOL 1

Lysis and sample clearing

- Thaw frozen cells at 0–6°C. Add 60 µl cold PBS, vortex.
- Add 10 µl (TLP) and 0.5 µl (CL), vortex.
- Incubate 2–3 min at 60°C with maximum agitation.
- Add 6 µl (CL) and 2 µl (R), vortex.
- Incubate 10 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)

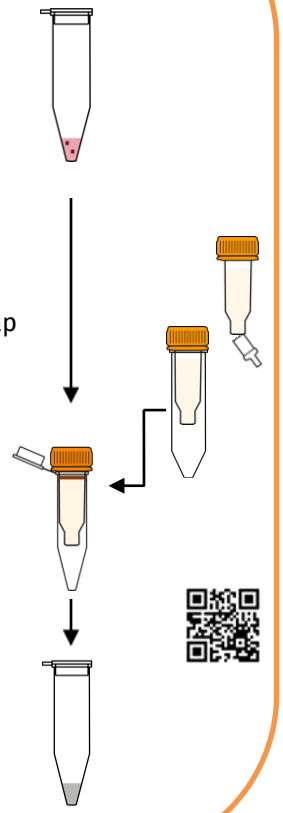
- Homogenize suspension by vortexing. Let stand for 20 min.
- Place in a 2 ml tube, punch a hole in the cap using the Cap Puncher, and break off bottom closure.
- Centrifuge 1 min at 750 x g.
- Place column in a 1.5 ml tube for elution.

Purification of lysate

- 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 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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