EchoLUTION Cell Culture DNA Kit - Protocol

for single-step purification of genomic DNA from cultured cells

This protocol has been developed for extraction of genomic DNA from up to 1x10^6 human or animal cells (cultured or primary cells).

Materials and equipment neededf

- **Up to 1x10^6 human or animal cells**
- **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 round per minute (rpm) into rcf
- **Thermal shaker** with **agitation** (for fastest performance), capable of heating to 60 °C. Alternatively: Heating Block or heat chamber
- **Vortexer**
- **Pipets** for 10 µL and 200 µL scale, 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
- 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 **2,000 x *g**** (cell pelleting) or **1,000 x g*** (spin column).
- Important: ***Switch to relative centrifugal force, rcf*** **(x *g****, not rpm).

BioEcho cap puncher

PROTOCOL 1: Purification **using** the Cap Puncher

Lysis

1. Harvest cells (up to 1x10^6 cells) by centrifugation at **2,000 x *g*** for **1 min** in a 1.5 mL reaction tube and remove supernatant carefully.
   Important: Make sure that the cell pellet is retained during this step.
2. Add **55 µL Lysis Buffer Cell DNA** (LB) and **25 µL TurboLyse Protease Cell DNA** (P) and resuspend cell pellet completely by pulse-vortexing.
   If working with more than two samples, prepare a **Lysis master mix** with a final volume that is 10 % larger than required for the number of samples.
   Note: The Lysis Buffer Cell DNA contains non-soluble grinding material that needs to be whirled up by vortexing before aspiration.

Lysis master mix:

No. of samples	1	6 (+10 %)	12 (+10 %)	Yours
(LB) Lysis Buffer Cell DNA (µL)	55	370	730	
(P) TurboLyse Protease Cell DNA (µL)	25	170	330	
Final volume (µL)	**80**	**540**	**1,060**	

3. Add **80 µL of the Lysis master mix** to the pellet. Resuspend cell pellet completely by pulse-vortexing.
4. Place the reaction tube(s) in the thermal shaker and incubate at **60 °C** for **10 min** with max. agitation (or for **15 min** if agitation is not feasible, in this case pulse-vortex 2 times during lysis).
   Note: Depending on cell type and number of cells, lysis step could be increased up to 30 minutes to optimize the results

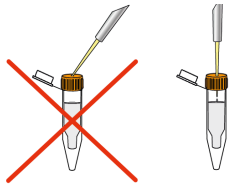
   Meanwhile during lysis, proceed with step 5, "Column preparation"

Column preparation (during step 4)

5. Vortex the **EchoLUTION Spin Column** briefly and place into a **2 mL** reaction tube. Let stand for 5-10 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 clockwise 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 sample DNA and place back into the rack.
   Continue with "Purification of DNA".

Purification of DNA

9. After having performed step 4, add **10 µL Clearing Solution Cell DNA** (CS) and **1 µL RNase Cell** (R) to each lysed sample and vortex **vigorously** with **four pulses of 10 sec each** until the sample is homogenized. The sample becomes cloudy.
   Note: Clearing Solution Cell DNA and RNase Cell may be mixed before addition.
10. Incubate for **2 min at room temperature** to remove RNA.
11. Centrifuge **for 2 min** at **max. 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 with pipette tip!
- Residual sample particles may be loaded and will not interfere with purification

13. Centrifuge for **1 min** at **1,000 *x g***. The purified genomic DNA elutes into the 1.5 mL elution tube and can be immediately applied in downstream applications.

The eluted DNA can be used immediately or kept at 2-8°C or for long-term storage at –20°C. For spectrophotometric analysis, use **Low-TE Buffer** (T) supplied with the kit as blank.

PROTOCOL 2: Purification **without** a Cap Puncher

Lysis

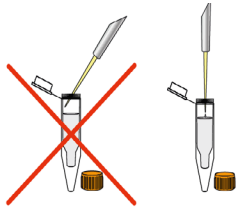
1. Perform steps 1-4 from PROTOCOL 1.

Column preparation (during step 3)

5. Vortex the **EchoLUTION Spin Column** briefly and place into a **2 mL** reaction tube. Let stand for 5-10 min.
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 **10 µL Clearing Solution Cell DNA** (CS) and **1 µL RNase Cell** (R) to each lysed sample and vortex **vigorously** with **four pulses of 10 sec each**. The sample becomes cloudy.
   Note: Clearing Solution Cell DNA and RNase Cell may be mixed before addition.
10. Incubate for **2 min** at **room temperature** to remove RNA traces.
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:



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!**

13. Centrifuge for **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 Cell Culture 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 matching the g-force using the formula: rpm = 1,000 x $\sqrt{(\frac{g}{1.12 \times r})}$, 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.

EchoLUTION Cell Culture DNA Kit

for single-step purification of genomic DNA from cell culture samples

Product no. (rxn's)	010-006-010 (10)	010-006-050 (50)	010-006-250 (250)
Kit contents	Lysis Buffer Cell DNA, TurboLyse Protease Cell DNA, Clearing Solution Cell DNA, RNase Cell, Low-TE Buffer and Spin Columns Cell DNA		

Quick PROTOCOL (please read protocol first)

Lysis

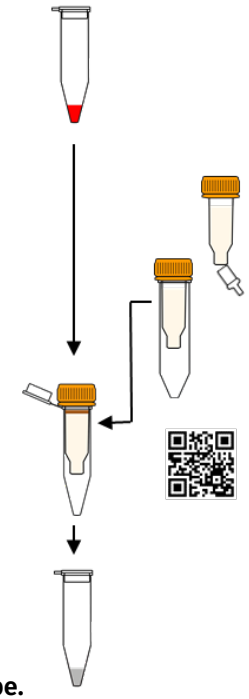
- Transfer up to **1x10⁶ cultured** cells to reaction tube.
- Centrifuge **1 min** at **2000 x g** to pellet cells.
- Resuspend cell pellet in **55 µL** (LB) + **25 µL** (P), resuspend pellet by pulse-vortexing.
- Incubate **10 min** at **60 °C,** max. agitation.
- Add **10 µL** (CS) + **1 µL** (R) and **pulse-vortex 4 x 10 sec.**
- Centrifuge **for 2 min** at **max. speed.**

Column preparation (during 60 °C incubation)

- Vortex **EchoLUTION column** and place in a **2 mL** tube. Let stand for 5-10 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.



2023/05/2

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