

# 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  $1 \times 10^6$  human or animal cells (cultured or primary cells).

## Materials and equipment needed

- **Up to  $1 \times 10^6$  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  $60^\circ\text{C}$ . Alternatively: Heating Block or heat chamber
- **Vortexer**
- **Pipets** for 10  $\mu\text{l}$  and 200  $\mu\text{l}$  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  $60^\circ\text{C}$ .
- Set the microcentrifuge to  **$2,000 \times g^*$**  (cell pelleting) or  **$1,000 \times g^*$**  (spin column).
- **Important:** Switch to **relative centrifugal force**, **rcf** ( $x g^*$ , not rpm).

## PROTOCOL 1: Purification using the Cap Puncher



BioEcho cap puncher

### Lysis

1. Harvest cells (up to  $1 \times 10^6$  cells) by centrifugation at  **$2,000 \times g$**  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  $\mu\text{l}$  Cell Lysis Buffer** (LB) and **25  $\mu\text{l}$  TurboLyse C Protease** (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 Cell Lysis Buffer 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) Cell Lysis Buffer ( $\mu\text{l}$ )       | 55        | 370        | 730          |       |
| (P) TurboLyse C Protease Mix ( $\mu\text{l}$ ) | 25        | 170        | 330          |       |
| <b>Final volume (<math>\mu\text{l}</math>)</b> | <b>80</b> | <b>540</b> | <b>1,060</b> |       |

3. Add **80  $\mu\text{l}$  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  **$60^\circ\text{C}$**  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  **$1,000 \times g^*$** . 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  $\mu\text{l}$  Clearing Solution** (CS) and **1  $\mu\text{l}$  RNase** (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 and RNase A 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  $\mu\text{l}$ )** 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  **$1,000 \times g$** . 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  $2-8^\circ\text{C}$  or for long-term storage at  $-20^\circ\text{C}$ . For spectrophotometric analysis, use **1x Tris Buffer** (T) supplied with the kit as blank.

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

### Lysis

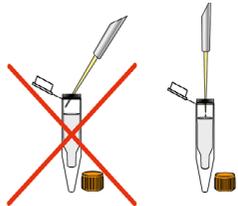
1. Perform steps 1-4 from PROTOCOL 1.

### 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 (CS)** and **1 µl RNase A (R)** to each lysed sample and vortex **vigorously** with **four pulses of 10 sec each**. The sample becomes cloudy.  
**Note:** Clearing Solution and RNase A 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:  $\text{rpm} = 1,000 \times \sqrt{\left(\frac{g}{1.12 \times r}\right)}$ , 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        | Cell Lysis Buffer, TurboLyse C Protease, Clearing Solution C, RNase A, 1x Tris Buffer, Spin Columns |                  |                   |

## Quick PROTOCOL (please read protocol first)

### Lysis

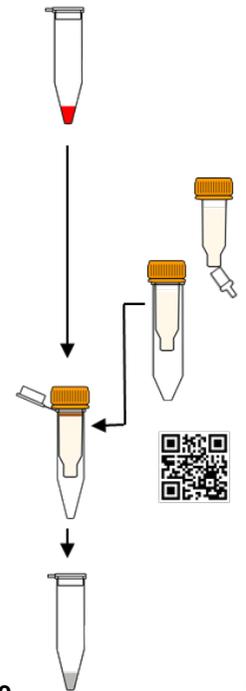
- Transfer up to **1x10<sup>6</sup> cultured cells** to reaction tube
- Centrifuge **1 min** at **2000 x g** to pellet cells
- Resuspend cell pellet in **55 µl (LB)** + **25 µl (P)**, resuspend pellet by pulse-vortexing
- Incubate **10 min** at **60°C**, max. agitation
- Add **10 µl (CS)** + **1 µl (R)** 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



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