EchoLUTION Cell Culture DNA 96 Kit - Protocol

for 96-well plate purification of genomic DNA from up to 2x10^6 human or animal cells (cultured or primary cells)

Materials and equipment needed:

Sample: up to 2x10^6 cultured or primary cells. Depending on culturing conditions (e.g. adherent, suspension), cells have to be transferred into the 96-well Lysis Plate.

Supplied with the kit:

- **Lysis Plate 96, Type 2:** 96-well plate for the lysis of the plant samples in a 96-well thermo shaker
- **Purification Plate 96 Type 1:** 96-well plate containing the matrix for DNA purification
- **Elution Plate 96 Type 1:** 96-well plate for the collection of the purified DNA
- **Sealing Foils** for sealing DNA eluate plate during storage
- **Adhesive Foils** for sealing Lysis Plate during lysis
- All needed reagents and solutions

Not supplied with the kit:

- **Conditioning Plate 96, Type 2**: 96-deep well plate with minimum of 800 µL well volume for the collection of void volume during preparation of **Purification Plate**. Reusable! BioEcho product no. 060-001-002-001
- **96-well swing-out centrifuge** (preferentially with switch option to rcf (x *g**))
  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
- **96-well plate thermal shaker** with **agitation** (for fastest performance), capable of heating to 60 °C and 80 °C. Alternatively: Heating Block or heat chamber
- **8-channel pipette** for 200 µL scale, corresponding wide bore pipet tips recommended.
- **Troughs** for master mix preparation holding up to 10 mL**.**
- **Plate(s) to be used as *Tara*** in the centrifuge in case an odd number of plates is processed.

Preparation before starting:

- Heat the thermal shaker, thermo block or heat chamber to 60 °C.
- Set the microcentrifuge to **1,000 x *g**.**

  Important: ***Switch to relative centrifugal force, rcf* (x *g****, not rpm).

PROTOCOL:

Lysis:

Important Note: It is highly recommended to perform the lysis step in the deep-well plate provided in this kit or in a similar plate format with minimal well volume of 0.5 mL. Please, transfer your cell samples into the Lysis Plate before adding the lysis master mix.

1. Transfer up to 2x10^6 cells per well into the **96-well Lysis Plate.**
   Note: Cell samples in a maximum volume of up to 400 µL each can be transferred into the **96-well Lysis Plate.**
2. Pellet cells by centrifugation at **2,000 x *g*** for **2 min** and remove supernatant carefully while leaving approximately **25-30 µL residual volume** in each well.
   Note: It is important **to not remove** cells from the cell pellet while removing the supernatant. Therefore, a pipetting robot or an appropriate pipetting aid is helpful to remove the supernatant, leaving the cell pellet in 25-30 µL liquid at the bottom of the well.
3. Prepare the **lysis master mix** for 96 lysis reactions with 20 % excess volume in reagent trough:
   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	**96** (incl.+20 %)	Yours
(P) TurboLyse Protease Cell DNA (µL)	25	2,880	
(LB) Lysis Buffer Cell DNA (µL)	55	6,340	
Final volume (µL)	**80**	**9,220**	

4. Add **80 µL lysis master mix** to the **Lysis Plate** containing the cell samples with an 8-channel pipette and resuspend the cell pellet by pipetting up and down. The total lysate volume per well is 105-110 µL.
5. Seal **Lysis Plate** with the **Adhesive Foil** (supplied with the kit).
6. Place the **Lysis Plate** in the thermal shaker and incubate at **60 °C** for **10 min** with max. agitation speed. Depending on the type of thermal shaker used, you may need to reduce the agitation speed to make sure to avoid wetting of the Adhesive Foil by the lysate during shaking.
   Note: Depending on cell type and number of cells lysis step could be increased up to 30 minutes to optimize the results.
   Note: If samples are **not** completely lysed after the time described above, continue with the next step. Residual cellular debris will not interfere with the purification performance.

Optional: After having performed step 6, add **1 µL RNase Cell** (R) to each well and incubate for 2 min at room temperature to remove RNA.

Meanwhile during lysis, proceed with step 7 of “Purification Plate Preparation” (below).

Preparation of Purification Plate (during step 6):

7. Carefully detach the **lower** and **upper** sealing foils from the **Purification Plate**.
   Note: If the Purification Plate was not shipped or stored upright, resin may stick to the upper foil. In this case, shake plate until resin is removed from upper sealing foil.
8. Plate preparation: Place the **Purification Plate** on top of a 96-deep well plate (“Conditioning Plate”, not supplied, minimum well volume of 800 µL) and centrifuge for **1 min** at **1,000 x *g**** to collect the void buffer from the purification resin in the **Purification Plate**. Discard the flow-through volume (“void volume”) collected in the lower **Conditioning Plate** (Conditioning Plate can be re-used).
9. Place the conditioned **Purification Plate** on top of an **Elution Plate** for elution of the purified DNA. Continue with “Purification of DNA”.

Purification of DNA:

10. Detach **Adhesive Foil** from the incubated **Lysis Plate** and **add 10 µL Clearing Solution Cell DNA** (CS) to each well of the **Lysis Plate** and mix by pipetting up down at least 10 times.
    The sample will become cloudy.
11. Centrifuge **Lysis Plate** for **3 min** at **full speed**.
12. Transfer the **lysis supernatant (max. 100 µL)** onto the **Purification Plate**.
    Important loading instructions:
    - Do not touch the cellular debris at the bottom of the well while removing the supernatant to avoid clogging of the pipet tip (preferentially, wide bore tips). Residual tissue particles may be loaded and will not interfere with purification.
    - During the loading step, make sure that the 8-channel pipette releases the lysate solution **slowly and vertically**, non-angular onto the middle of the resin surface!
    - Do not punch pipette tip into the resin bed during loading of lysate!
13. After completion of the loading step, centrifuge **Purification Plate** on top of an **Elution Plate** as “plate sandwich”.
14. Centrifuge for **1 min** at **1,000 x *g****.
15. The purified DNA elutes into the **Elution Plate** and can be immediately applied in downstream applications.

The eluted DNA can be used immediately or stored at 4 °C or for long-term storage at −20 °C. For spectrophotometric analysis, use **Low-TE Buffer** (T) supplied with the kit as blank.

Product use limitation:

The EchoLUTION CellCulture DNA 96 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.

EchoLUTION CellCulture DNA 96 Kit

for 96-well plate purification of genomic DNA from human or animal cells

Product no. (plates)	010-106-002 (2 x 96)	010-106-008 (8 x 96)
Kit contents	Lysis Plate 96 Type 2, Purification Plate 96 Type 1, Elution Plate 96 Type 1, Adhesive Foils, Sealing Foils, Lysis Buffer Cell DNA, TurboLyse Protease Cell DNA, RNase Cell, Clearing Solution Cell DNA, Low-TE Buffer	
Related products	Conditioning Plate 96 Type 2; 060-001-002-001	

Quick PROTOCOL (please read protocol first)

Sample Lysis and Clearing

- Load **Lysis Plate** with suspended cell samples.
- Centrifuge **Lysis Plate** for **2 min** at **2,000 x *g****.
- **Remove** supernatant and leave **25-30 µL** liquid in the well without touching the cell pellet.
- Prepare **lysis master mix** for 96 rxns. + 20 % vol. excess.
- Add **80 µL lysis master mix** per well.
- Seal **Lysis Plate** tight with the **Adhesive Foil.**
- Incubate the **Lysis Plate** for **10 min at 60 °C** with max. agitation.
- Detach **Adhesive Foil** and add **10 µL** of (CS) per well of the **Lysis Plate** and mix by pipetting up & down.
- Centrifuge **Lysis Plate** for **3 min** at **full speed.**

Preparation of Purification Plate (during 60 °C incubation)

- Detach lower and upper foil from **Purification Plate.**
- Place **Purification Plate** on top of a **Conditioning Plate** (deep well).
- Centrifuge **1 min** at **1,000 *x g**** to elute void buffer from **Purification Plate.**
- Place prepared **Purification Plate** on top of **Elution Plate.**

Purification of DNA

- Transfer **lysis supernatant (max. 100 µL)** to **Purification Plate.**
- Centrifuge **1 min** at **1,000 *x g**** to elute DNA into **Elution Plate.**
- Eluted DNA is ready to use.

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