# EchoLUTION™ Tissue DNA Micro Kit

## Spin column kits

# USER MANUAL

REF

010-002-010

010-002-050

010-002-250

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# 1. INTENDED PURPOSE

The BioEcho EchoLUTION Tissue DNA Micro Kit is intended for easy, rapid, and efficient genomic DNA (gDNA) extraction from mammalian tissue samples in 50 minutes. The excellent yield and purity of tissue DNA obtained with the EchoLUTION Tissue DNA Micro Kit allows use in downstream applications without further processing.

The EchoLUTION Tissue DNA Micro Kit is intended for research use only.

# 2. EXPLANATION OF THE KIT

The EchoLUTION Tissue DNA Micro Kit is characterized by the EchoLUTION single-step purification technology and a tailored lysis. Together, these steps reduce the procedure to 50 minutes with consistent sensitivity compared to established methods.

The EchoLUTION Tissue DNA Micro Kit benefits are:

- Short processing time
- Fast lysis
- Few protocol steps
- High sample throughput with minor equipment and capital investment
- 70 % less plastic waste
- Less toxic reagents

For further details about kit specifications, see Table 1.

*Table 1:* ***Kit specifications***

| Specification | Description | |
|---|---|---|
| **Sample input** | Fresh and frozen tissues | |
| **Sample condition** | Fresh, frozen, or stabilized | |
| **Tissue type** | Standard | 10 mg |
| | High DNA content (e g., spleen, liver, kidney) | 5 mg |
| | Low DNA content (e. g., muscle, cartilage) | 20 mg |
| **Purified nucleic acid** | Genomic DNA | |
| **Elution volume** | 100 µL | |
| **Expected yields** | 5–12 µg (depends on amount of starting material) | |

## 2.1. *Single-step purification principle*

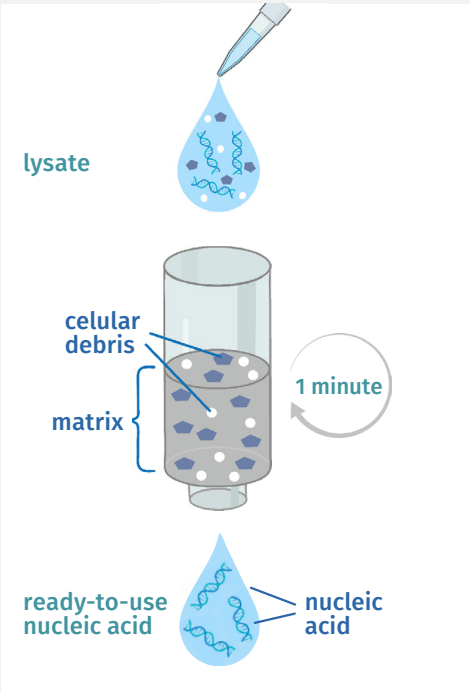
All BioEcho nucleic acid extraction kits are based on the EchoLUTION technology, which consists of tailored sample processing that includes lysis followed by single-step purification. The purification step works differently than conventional methods such as magnetic-bead and silica kits based on the bind–wash–elute method, and therefore needs only one centrifugation step.

The lysate is transferred onto the Spin Column or Purification Plate.

In a one-minute centrifugation step, nucleic acids pass through the purification matrix without interaction.

Impurities are held back and, thereby completely removed.

The nucleic acids are in the flow-through and ready-to-use.



## 2.2. General comments

***Comparison of the EchoLUTION technology to silica technologies—general aspects and handling***

Using the EchoLUTION technology, nucleic acids are not bound to a membrane or magnetic beads and can migrate freely through the filter matrix. Unwanted components of the lysate are removed from the sample by remaining in the purification matrix.
The advantages of the EchoLUTION technology are:

1. No time-consuming washing steps
2. Easy handling
3. Reduced plastic waste

In contrast, silica technologies are based on the principle of concentration. Here, the nucleic acids present in the lysate bind to a silica surface (membrane, magnetic beads), while unwanted cell components are removed by repeated washing with chaotropic and alcohol-containing wash buffers. Eventually, the nucleic acids are eluted with an aqueous buffer in the desired volume. Due to the repeated washing steps, silica-based methods are time-consuming, labor-intensive, and environmentally unfriendly.

***Handling of purification matrix***

The EchoLUTION purification matrix must be kept undamaged to avoid short-circuit currents. Short-circuit currents result in the introduction of lysis components into the eluate and inadequate purification, which can lead to inhibition in downstream analysis. Therefore, when applying the lysate to the column, **avoid touching the surface** of the filter matrix, and pipet **the sample very slowly (ideally dropwise)**.

To guarantee proper handling of the chromatography columns, be sure to use the recommended *g*-force centrifuge settings. Most centrifuges offer the choice between rpm and *g*-force (rcf); if rcf is not available, calculate the rpm corresponding to the required *g*-force using the calculator in the link or the QR code below:


http://www.geneinfinity.org/sp/sp_rotor.html

**For support on suitable centrifuges, please contact us.**

### *Handling DNA*

In general, sterile microbiological practices should always be used when working with DNA. The most common sources of contamination are dust and hands, as they can hold bacteria and molds. Therefore, pay attention to what you're doing and always wear gloves while handling reagents and samples. Replace gloves regularly and keep tubes closed when possible.

Bench surfaces, laboratory equipment (e.g., pipets and electrophoresis tanks) and non-disposable plasticware can be decontaminated using general laboratory reagents. Plasticware can be decontaminated with commercially available DNase-removing solutions.

It is recommended to store the purified DNA in a tightly capped tube at 2–8 °C for 24 hours or at –20 °C for long-term storage.

### *Input material*

Best results are obtained with fresh material or material that has been immediately frozen after sampling and stored at –20 °C. Repeated freezing and thawing of samples should be avoided, since this leads to reduced DNA fragment size. Stabilized tissue samples (e.g., with BioEcho PurifyLater Tissue Stabilizer; product number: 030-002-100) can be stored in a refrigerator, according to the manufacturer's instructions.
In general, be aware that the use of low-quality starting material leads to reduced length and yield of purified DNA.

### *Lysing and homogenizing starting material*

The efficient lysis and homogenization of the starting material is an asset for successful extraction of DNA from tissues.

Correct lysis of the tissue is necessary to release all the DNA contained in the sample. Incomplete lysis results in drastically reduced DNA yields. Make sure to cut your tissue into small pieces to ensure the best lysis conditions. After completing the lysis, check the sample for visible clumps. If clumps remain in the sample, prolong the incubation time. For difficult samples, incubation overnight is possible.

The homogenization of the sample is also important to reduce the viscosity of the lysates, to ensure precise handling and pipetting. Clumps remaining after lysis can lead to reduced DNA yield and handling issues.

# 3. MATERIALS

## 3.1. Materials provided

*Table 2:* ***Content of EchoLUTION Tissue DNA Micro Kit***

| Product number | 010-002-010 | 010-002-050 | 010-002-250 |
|---|---|---|---|
| **Product name** | **EchoLUTION Tissue DNA Micro Kit (10)** | **EchoLUTION Tissue DNA Micro Kit (50)** | **EchoLUTION Tissue DNA Micro Kit (250)** |
| **Reactions** | 10 | 50 | 250 |
| **Lysis Buffer Tissue DNA (LB)** | 1 mL | 5 mL | 13 mL |
| **TurboLyse Protease Tissue DNA (P)** | 60 µL | 300 µL | 1.5 mL |
| **RNase Tissue (R)** | 10 µL | 50 µL | 250 µL |
| **Clearing Solution Tissue DNA (CS)** | 100 µL | 500 µL | 2.5 mL |
| **Low-TE Buffer (T)** | Blank only | Blank only | Blank only |
| **Spin Columns Tissue DNA** | 10 | 50 | 250 |

## 3.2 Materials required but not provided

### A. Microcentrifuge tubes

Use a 2 mL tube for Spin Column preparation and a 1.5 mL tube for sample lysis and elution.

## 3.3 Optional materials

### A. BioEcho Cap Puncher

Alternatively, the Cap Puncher can be used for convenient handling of Spin Columns. To purchase this item, use the product number 050-001-001.

### B. Spin Column Adapter for Plate Centrifuges

If you want to use a plate centrifuge for Spin Columns and avoid the standing time of the Spin Columns mentioned in the protocol, we suggest using a swing-out rotor centrifuge with our Spin Column Adapter for Plate Centrifuges (product number 050-011-024)

### 3.4 *Laboratory equipment needed*

***A. Microcentrifuge***

Centrifugation can be performed in a microcentrifuge with a rotor for 2 mL reaction tubes. The centrifuge must be capable of reaching 3,000 x *g*. When using a plate centrifuge, please use the Spin Column Adapter for Plate Centrifuges offered by BioEcho (product number: 050-011-024).

***B. Pipetting equipment***

Pipetting can be performed using a single-channel pipette. Pipette tips with filters are recommended.

***C. Thermal shaker***

The thermal shaker used for the lysis step must be capable of heating to 80 °C and shaking at 1,400 rpm (e.g., Eppendorf® C Thermomixer). Alternatively, you can use a heating block or heat chamber.

***D. Vortex mixer***

A vortex mixer is required for mixture of solutions.

***E. Ceramic blade scalpels***

BioEcho offers a scalpel suitable for cutting tissue material (e.g., product number 050-002-001).

## 4. STORAGE AND STABILITY

### 4.1. *Kit and reagents*

- The EchoLUTION Tissue DNA Micro Kit is shipped at ambient temperature.
- Upon kit arrival, the Enzyme Box should be stored at 2–8 °C. The other kit components are stable at room temperature (15–25 °C).

### 4.2. *Sample collection*

In general, be aware that the use of low-quality starting material leads to DNA fragmentation and reduced yield of purified DNA.

Make sure to adjust starting material weight according to protocol requirements, as stated in Table 1 “Kit specifications” and section 2 “Explanation of the kit”.

***A. Handling frozen tissues for DNA extraction***

The best extraction results are obtained with fresh samples. If samples cannot be processed freshly, freeze tissue immediately and store at –20 °C. Avoid freezing and thawing the samples repeatedly, as this can affect DNA integrity. When processing frozen samples, cut the frozen samples into small pieces, and proceed immediately with extraction protocol step 2.

***B. Handling stabilized tissues for DNA extraction***

Stabilized tissue samples (e.g., with BioEcho PurifyLater Tissue Stabilizer; product number: 030-002-100) can be stored in a refrigerator according to manufacturer's instructions. Make sure to rinse the tissue samples briefly with water to remove traces of stabilization solution, as they can interfere with kit performance. After rinsing, cut the samples into small pieces, and proceed immediately with extraction protocol step 2.

## *4.3. Storage and stability of purified nucleic acids*

It is recommended to store purified DNA in a tightly capped tube at 2–8 °C for a maximum of 24 hours or at –20 °C for long-term storage.

# 5. WARNINGS AND SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please refer to the instructions supplied with our safety data sheets (SDS). Please contact us for the SDS.

*Table3:* ***EchoLUTION Tissue DNA Micro Kit safety information***

| Component | Hazardous component | GHS symbol | Hazard statements | Precautionary statements | Additional statement |
|---|---|---|---|---|---|
| Lysis Buffer Tissue DNA (LB) | Anionic detergent |  Danger | H315; H318 | P261; P280 P305+P351+P388 | – |
| TurboLyse Protease Tissue DNA (P) | Microbial Protease |  Danger | H315, H317; H319; H334; H335 | P101; P102; P103; P261; P280 P305+P351+P388 P342+P311 P405; P501 | – |
| Clearing Solution Tissue DNA (CS) | Strontium chloride |  Danger | H315; H319; H335 | P261; P280 P305+P351+P388 | – |

***Hazard Statements***

H315: Causes skin irritation.
H317: May cause an allergic skin reaction.
H318: Causes serious eye damage.
H319: Causes serious eye irritation.
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335: May cause respiratory irritation.

***Precautionary statements***

P101: If medical advice is needed, have product, container label or MSDS at hand.
P102: Keep out of reach of children.
P103: Read label and MSDS before use.
P261: Avoid breathing dust/fume/gas/mist/vapors/spray.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305+ P351+P388: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P342+P311: If experiencing respiratory symptoms: Call a POISON CENTER or doctor.
P405: Store locked up.
P501: Dispose of contents/container in accordance with local/regional/national/international regulations.

# 6. DISPOSAL

Please follow local regulations regarding the collection and disposal of hazardous waste. Contact your waste disposal company to obtain information on laboratory waste disposal (waste code number 16 05 06). For further information, please refer to the instructions supplied with our SDS. Please contact us for the SDS.

Dispose of biological samples as biohazardous waste and all remaining liquid waste generated during the purification procedure according to local regulations.

***A. Components and Spin Columns***

No special measures for disposal are necessary. Components that have come into contact with potentially infectious material should be autoclaved. Used components may retain some buffer residues, and should be disposed of according to local /regional/national/international regulations.

# 7. PROTOCOL

This protocol has been developed for purifying genomic DNA from fresh, frozen, and stabilized mammalian tissue samples, including DNase-rich (e.g., spleen, liver, kidney) and lipid-rich tissues (e.g., brain, fat), using the EchoLUTION Tissue DNA Micro Kit.

This is not a silica-based kit. Please read the instructions carefully before starting!

***Preparation before starting:***

- Vortex EchoLUTION Spin Columns thoroughly to homogenize the purification matrix and remove air bubbles. If necessary, flick or gently spin down by hand until it is free of air bubbles. Place each column in a 2 mL reaction tube (not provided) and let them stand to sediment the matrix for at least 10 minutes.
- Prepare 1.5 mL microcentrifuge tubes (not provided, preferably safe lock) for each sample.
- Pre-heat the thermal shaker to 60 °C.
- Set microcentrifuge to 1,000 x *g*.

***IMPORTANT NOTE:*** *Choose x g (RCF), NOT RPM, unless stated otherwise.*

***1. Preparation of tissue samples***

***a) Fresh tissue***

- Cut the tissue sample into small pieces.
- Transfer the tissue sample to the bottom of a 1.5 mL reaction tube that is cooled on ice (or cooling block) to avoid DNA degradation during sample loading.

***b) Frozen tissue***

- Cut the frozen tissue sample into small pieces.
- Transfer the tissue sample to the bottom of a 1.5 mL reaction tube that is cooled on ice (or cooling block) to avoid DNA degradation during sample loading.

***c) Stabilized tissue***

- Rinse tissue samples briefly with water to remove traces of stabilization solution.
- Cut the tissue sample into small pieces.
- Transfer the tissue sample to the bottom of a 1.5 mL reaction tube that is cooled on ice (or cooling block) to avoid DNA degradation during sample loading.

***NOTES:***

- *If possible, cut tissue into small pieces to ensure best lysis conditions.*
- *Make sure you use no more than the maximum sample amount stated in section 2, Table 1.*

## 2. *Lysis of tissue samples*

- Prepare a lysis master mix for the number of reactions with 20 % excess volume in a suitable vessel. Each tissue sample requires 90 µL Lysis Buffer Tissue DNA (LB) and 5 µL TurboLyse Protease Tissue DNA (P). Mix by flicking or vortexing and transfer 95 µL to each tissue sample.

*Table 4:* ***Lysis master mix***

| **Number of samples** | **1** | **12 x (+20 %)** | **Your calculation** |
|---|---|---|---|
| Lysis Buffer Tissue DNA (LB) | 90 µL | 1296 µL | |
| TurboLyse Protease Tissue DNA (P) | 5 µL | 72 µL | |
| **Final volume** | 95 µL | 1368 µL | |

- Incubate the lysate at 60 °C in a thermal shaker with constant shaking at 1,400 rpm for 30 minutes.
- Incubate samples at 80 °C for 10 minutes with constant shaking at 1,400 rpm.
- Allow the samples to cool to room temperature for 5 minutes before proceeding with step 4.

***NOTES:***

- *During incubation, proceed with step 3 "Spin Column Preparation".*
- *Depending on the tissue type, the lysis time can vary (from 15 minutes to overnight). Make sure to cut the tissue into small pieces to speed up the process. Check during the incubation to see if cellular debris is present and prolong the incubation time if needed. Cellular debris do not interfere with the purification process, but can lower the DNA yield, as not all DNA is released from the tissue.*
- *Ideally, incubating samples should be shaken at 1,400 rpm; using a lower rpm might require a longer incubation.*

## 3. *Spin Column preparation*

- Loosen the cap of the Spin Column half a turn and snap off the bottom.
- Place the Spin Column in a 2 mL reaction tube (not provided).
- Centrifuge 1 minute at 1,000 x *g* and discard flow-through.
- Place the Spin Column in a fresh 1.5 mL reaction tube (not provided).

***ALTERNATIVELY:***

*You can use the BioEcho Cap Puncher to open the column (not supplied, product number 050-001-001). To use the Cap Puncherr correctly, punch a hole in the column cap, and lift the column together with the Cap Puncher out of the 2 mL reaction tube. Snap off the bottom closure of the column and detach the Cap Puncher. Place the punched Spin Column back into the 2 mL reaction tube. Centrifuge 1 minute at 1,000 x g, discard flow-through and place the Spin Column in a fresh 1.5 mL reaction tube.*

### *4. DNA purification*

- After cooling the samples to room temperature, add 10 µL Clearing Solution Tissue DNA (CS) to the lysate and vortex briefly.
- Optional: Add 1 µL RNase Tissue (R) with the Clearing Solution Tissue DNA (CS) to each lysed sample, vortex 3 seconds and incubate for 2 minutes at room temperature to remove traces of RNA.


- Centrifuge for 2 minutes at maximum speed.
- Open the Spin Column and transfer up to 100 µL supernatant slowly to the prepared Spin Column.

  ***IMPORTANT NOTE:***

  - *Pipet slowly, drop-by-drop, and vertically onto the middle of the Spin Column to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading!*
  - *When using the BioEcho Cap Puncher, the supernatant can be pipetted into the column without removing the cap first by using the hole in the tube cap.*

- Close the cap of the Spin Column and loosen the cap again half a turn.


- Centrifuge the loaded Spin Column for 1 minute at 1,000 x *g*.
- Purified DNA is in the flow-through.

The eluted DNA can be used immediately or stored at 2–8 °C or, for long-term storage, at–20 °C.

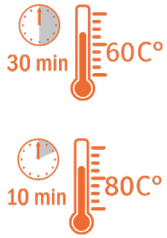
***IMPORTANT NOTE:***

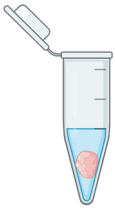
*For spectrophotometric analysis, use the Low-TE Buffer (T) supplied with the kit as a blank.*

## 7.1. Quick protocol EchoLUTION Tissue DNA Micro Kit

***IMPORTANT NOTE:*** *Please use the quick protocol only after you have read and understood the complete user manual.*

### 1. Lysis

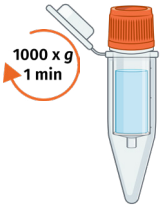


- Transfer tissue sample to a 1.5 mL tube.
- Add 90 µL LB and 5 µL P and vortex shortly.
- Incubate at 60 °C for 30 min, 1,400 rpm.
- Incubate at 80 °C for 10 min, 1,400 rpm.
- Cool down.

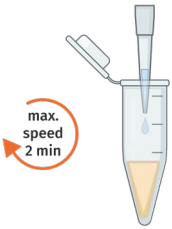
### 2. Spin Column preparation

- Loosen the cap of the Spin Column half a turn and snap off the bottom.




- Place Spin Column in a 2 mL tube.
- Centrifuge 1 min at 1,000 x *g*.
- Discard flow-through.
- Place Spin Column in a new 1.5 mL tube.

### 3. DNA purification




- Add 10 µL CS and mix.
- Optional: Add 1 µL RNase with the CS, mix and incubate for 2 min at RT.
- Centrifuge for 2 min at max. speed


- Transfer 100 µL lysate. Pipet slowly, drop-by-drop onto the middle of the column without touching the matrix.
- Centrifuge 1 min at 1,000 x *g*.
- Purified DNA is in the flow-through.

## 8. QUALITY CONTROL

Following the BioEcho Quality Management System, each lot of the EchoLUTION Tissue DNA Micro Kit is tested against predetermined specifications to ensure consistent product quality.

## 9. TROUBLESHOOTING

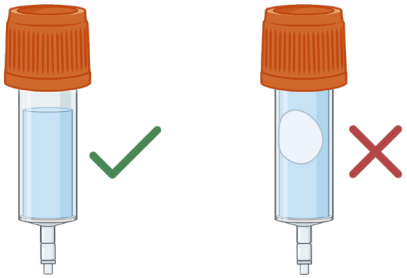
| Observation | Comments and suggestions |
|---|---|
| **DNA yield and concentration is low** | **Tissue content used had low DNA content**<br>Some tissues have very low DNA content. Also, the storage conditions can lead to variations in DNA content and to high amounts of degraded genomic DNA.<br><br>**Sample input**<br>Always use the correct weight of sample input to ensure appropriate experimental conditions. Exceeding the maximum amount of tissue will compromise the purity of the extracted DNA. Matrix overloading can lead to a milky eluate and carryover of debris.<br><br>**Incomplete sample disruption**<br>Insufficient sample disruption and homogenization lead to inefficient sample lysis and low DNA yield. Please make sure to cut your tissue samples into small pieces to ensure best lysis conditions.<br><br>**Insufficient sample lysis**<br>Incomplete lysis of tissue samples can lower the DNA yield, as not all DNA is released from the tissue.<br><br>TurboLyse Protease Tissue DNA (P) needs to be stored at 2–8 °C. If TurboLyse Protease Tissue DNA (P) was stored at higher temperatures for a prolonged time, repeat the procedure using new samples and fresh TurboLyse Tissue Protease Mix (product number: 010-122-001 or 010-122-020). |

| | |
|---|---|
| **DNA yield and concentration is low (continuation)** | Depending on the tissue type, the lysis time can vary from 15 minutes to overnight. During the incubation, check to see if cellular debris are present, and prolong the incubation time if needed. Be sure to check the incubation regularly, as too long digestion can decrease the DNA yield.<br><br>Some tissues are easy to lyse (e.g., spleen, liver, kidney). Difficult tissues to lyse (e.g., muscle, fatty tissues and cartilage) need longer incubation times.<br><br>**Loading of column**<br>Correct loading of the column is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the column to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading.<br><br>**Centrifuge settings**<br>Most centrifuges offer the choice between rpm and *g*-force (rcf); if not, calculate the rpm, see section 2.2. Always make sure to stick to the correct time mentioned in the protocol, to avoid insufficient passage through the matrix bed. |
| **$A_{260}/A_{280}$ and $A_{260}/A_{230}$ values are low** | **Wrong blank in measurements**<br>Use supplied Low-TE Buffer (T) as a blank in measurements.<br><br>**Sample input**<br>Always use the correct amount of tissue to ensure appropriate experimental conditions, and do not load more than 100 µL onto the matrix. Depending on the sample material, the matrix can be loaded with up to 100 µL. Overloading the matrix will compromise the sample purity. |

| | |
|---|---|
| **Degraded DNA** | **Incorrect storage of samples**<br>Extension of the storage time can lead to DNA degradation. Best results are obtained with fresh material or material that has been immediately frozen and stored at –20 °C. Repeated freezing and thawing of samples should be avoided, since this leads to reduced DNA fragment size. Stabilized tissue samples (with, e.g., BioEcho PurifyLater Tissue Stabilizer; product number 030-002-100) can be stored in a refrigerator according to manufacturer 's instructions. Be sure to check the lysis incubation regularly as too long digestion can decrease the DNA quality.<br><br>**Contamination with nucleases**<br>Even though materials and solutions included in the kit are nuclease-free, nucleases can be introduced when handling the samples. Check all equipment, including pipettes, pipette tips, microcentrifuge tubes, etc., for contamination, and use the appropriate precautions during the entire extraction procedure. See section 2.2 for detailed instructions. |
| **Eluate contains RNA residues** | **Optimal RNase procedure not used**<br>Although RNase digestion is not necessary for most tissue samples, RNA-rich tissues might require RNase digestion to eliminate RNA. In these cases, prepare a master mix with RNase Tissue (R) and Clearing Solution Tissue DNA (CS) in step 4 "DNA purification" of the protocol. If RNA is still present in the sample after digestion, prolong the incubation period. |
| **Air bubbles in Spin Columns** | **Inappropriate homogenization**<br>To homogenize the matrix in the Spin Columns, the Spin Columns need to be vortexed thoroughly. You can perform this step in an upright position, on the side, or upside down depending on what works best for you. If air bubbles are visible (see picture), it is mandatory to remove them completely. |

**Air bubbles in Spin Columns (continuation)**

To remove air bubbles, flick or gently spin down by hand until it is free of air bubbles or quickly vortex again in an upright position at the end. Place each column in a 2 mL reaction tube (not provided) and let them stand to sediment the matrix until used.

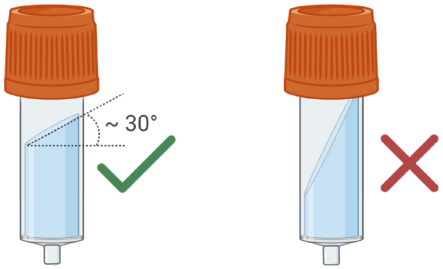


**Highly tilted matrix in column**

**Inappropriate handling of Spin Columns**

A highly tilted matrix after conditioning leads to insufficient interaction time between the DNA and the matrix, which can result in poor extraction performance. However, when using fixed-angle rotor centrifuges, the matrix does not become fully horizontal after conditioning. A slightly tilted surface according to the angle of the rotor (usually 30°) is to be expected, and this does not limit the purification ability.

But if you observe that the matrix is tilted to a higher angle (see picture below), we recommend prolonging the standing time after resuspending the column before conditioning. For quicker processing, we recommend to vortex the Spin Columns upon receipt of the kit and storing them in 2 mL microcentrifuge tubes in an upright position till used.



| | |
|---|---|
| **Highly tilted matrix in column (continuation)** | Alternatively, if you want to avoid prolonging the standing time, we suggest using a swing-out rotor centrifuge with our Spin Column Adapter for Plate Centrifuges (product number 050-011-024).<br><br>Another reason could be that the Spin Column was completely closed during centrifugation and a vacuum was generated. |
| **Poor performance in downstream experiments** | **Loading of Spin Columns**<br>The correct loading of the column is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the column to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading.<br><br>**Centrifuge settings**<br>Most centrifuges offer the choice between rpm and *g*-force (rcf); if rcf is not available, calculate the rpm, see section 2.2. Always make sure to stick to the correct time mentioned in the protocol to avoid insufficient passage through the matrix bed. |
| **Occurrence of cross-contamination** | **Contaminated pipettes**<br>The use of contaminated pipettes can lead to cross-contaminations. BioEcho recommends a separate set of pipettes for sample and PCR preparation. These pipettes should be cleaned thoroughly at regular intervals. It is also recommended to use filter tips for all pipetting steps involving samples.<br><br>**Handling of samples**<br>Throughout the entire procedure, we recommend using microbiological, sterile practices. Pay attention and wear gloves while handling reagents and samples. The use of pipette tips with filters is recommended. |

| | |
|---|---|
| **Flow-through is missing or volume too low** | **Inappropriate handling of Spin Column**<br>The Spin Column was closed during centrifugation, and a vacuum was generated.<br><br>**Centrifuge settings**<br>Most centrifuges offer the choice between rpm and g-force (rcf); if rcf is not available, calculate the rpm, see section 2.2. Always make sure to use the correct time mentioned in the protocol to avoid insufficient passage through the matrix bed. |

**For questions and further troubleshooting, please contact us!**

## 10. LIMITATIONS OF USE

Limitations regarding EchoLUTION Tissue DNA Micro Kit are listed below.

- Strict compliance with the user manual is required for DNA purification. Following good laboratory practices is crucial for the successful use of the product. Appropriate handling of the reagents is essential to avoid contamination or impurities.
- The DNA yield varies and is dependent on several factors including the technique of the person taking the sample.
- The proof of principle for the EchoLUTION Tissue DNA Micro Kit was evaluated and confirmed using state-of-the-art PCR. Performance parameters are highly dependent on the quality of sample collection.
- The EchoLUTION Tissue DNA Micro Kit is for research use only.

## 11. SYMBOLS

The following table describes the symbols that appear on the labeling of the EchoLUTION Tissue DNA Micro Kit products and in this user manual.

*Table 5:* ***EchoLUTION Tissue DNA Micro Kit symbols***

| Symbols | Description |
|---|---|
|  | Manufacturer |
|  | For research use only |
|  | Product number |
|  | Batch code |
|  | Contains sufficient for < n > reactions |
|  | Temperature limitation |
|  | Do not re-use |
|  | Expiration date |
|  | Consult instructions for use |

# WE ARE INTERESTED IN YOUR EXPERIENCE WITH BIOECHO PRODUCTS!

**With questions or suggestions or for further troubleshooting, please contact us.**

Visit our website and shop for further information, tutorials, and application notes.

This user manual can be found in our shop on the corresponding product page.

Interested in publishing an application note with us? Please get in touch!

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