EchoLUTION™ FFPE DNA Kit

Spin column kits

USER MANUAL

REF

010-005-010

010-005-050

010-005-250

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1. INTENDED PURPOSE

The BioEcho EchoLUTION FFPE DNA Kit is intended for easy, fast, and efficient genomic DNA extraction from formalin-fixed paraffin-embedded (FFPE) tissue. The high yield and purity of total DNA obtained with the EchoLUTION FFPE DNA Kit allows use in several downstream applications, such as NGS, PCR, and qPCR without further processing.

The EchoLUTION FFPE DNA Kit is intended for research use only.

2. EXPLANATION OF THE KIT

The EchoLUTION FFPE DNA Kit is characterized by a tailored decrosslinking and lysis step followed by a single-step purification. Together, these steps reduce the overall extraction time drastically with consistent quality compared to state-of-the-art methods.

The key steps of the EchoLUTION FFPE DNA Kit procedure are:

1. FFPE decrosslinking
2. FFPE tissue lysis and removal of detergent and paraffin
3. Single-step DNA purification (see section 2.1)

In contrast to conventional methods, the benefits of the EchoLUTION FFPE DNA Kit are:

- Shorter processing time
- Fewer protocol steps
- Minor equipment and capital investment
- Less plastic waste
- Fewer toxic reagents (no usage of xylene or similar reagents during deparaffinization, no ethanol steps, in total less liquid waste)

For further kit specifications, see Table 1.

Table 1: ***Kit specifications***

Specification	Description
Sample type	FFPE tissue
Sample input	100–800 mm^2 area (thickness 10 µm) or 1–15 mg FFPE tissue
Sample condition	Formalin-fixed paraffin-embedded
Purified nucleic acid	DNA
Elution volume	~80 µL
Expected yields	Highly dependent on amount of starting material and tissue type

2.1. *Single-step purification principle*

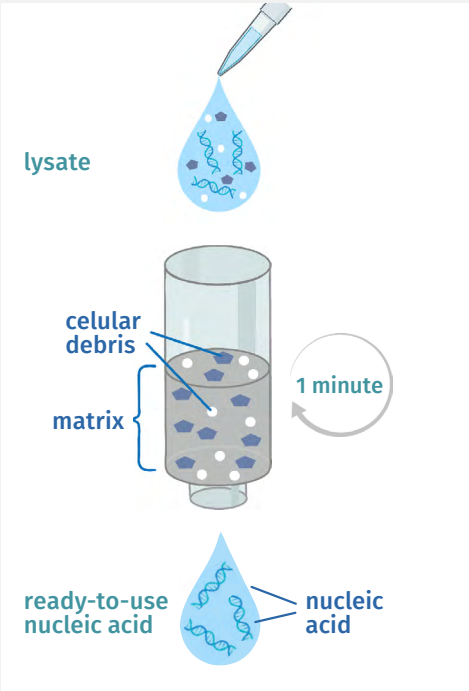
All BioEcho nucleic acid extraction kits are based on the EchoLUTION technology, which consists of tailored sample processing that includes lysis followed by single-step purification. The purification step works differently than conventional methods such as magnetic-bead and silica kits based on the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

The lysate is transferred onto the spin column or 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 EchoLUTION technology to silica methods—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 within the Spin Columns 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)**.
For proper handling of the Spin Columns, use the recommended *g*-force centrifuge settings. Most centrifuges offer the choice between rpm and *g*-force (rcf). If your centrifuge does not provide a *g*-force setting, you can calculate the rpm corresponding to the required *g*-force using the calculator in the link below or the QR Code:

www.geneinfinity.org/sp/sp_rotor.html

For support on suitable centrifuges, please contact us.

FFPE sample storage

In general, FFPE blocks can be stored at room temperature. For best DNA extraction results, samples should be stored at 2–8 °C or -20 to –80 °C.

Preparation of FFPE samples

Formalin fixation and paraffin embedding is a widely used and valuable process to preserve tissues. Many fixation protocols are available to match the wide range of applications in which FFPE tissue is used. Accordingly, there is no standard fixation protocol. However, the method used has a large influence on the quality of extracted nucleic acids and their performance in downstream applications. Several factors influence the tissue and quality of preservation. The

larger the biopsy, the more inhomogeneous the tissue preservation is likely to be. The amount of time after the biopsy is taken and before it is embedded in paraffin is also variable. Longer times before embedding can lead to degradation of proteins and nucleic acids contained in the tissue. In addition, the longer a biopsy is preserved in formalin, the greater is the degree of crosslinking. Crosslinking can be reversed with incubation at 90 °C. In the EchoLUTION FFPE DNA Kit protocol, the sample is incubated for 40 minutes. For FFPE tissue with a high degree of crosslinking, extending the incubation to 60 minutes can lead to more efficient decrosslinking and, therefore, in higher yields. However, it should be kept in mind that prolonging the 90 °C incubation can cause higher fragmentation of the DNA.

DNA fragmentation

Formalin fixation and paraffin embedding crosslinks cellular components such as DNA, RNA, proteins, and enzymes to stop cell metabolism. The longer the FFPE sample is stored, the higher the degree of the resulting nucleic acid fragmentation. Furthermore, fragmentation happens at a higher rate at higher temperatures. Thus, on one hand, higher temperatures during the embedding process as well as during the decrosslinking step increase the degree of fragmentation. On the other hand, these higher temperatures can increase the nucleic acid yield. Therefore, you must balance the advantages and disadvantages according to your desired result. For optimal DNA extraction, the time of the decrosslinking incubation should take into consideration the sample age and length of formalin fixation, both of which also have an impact on fragmentation. For samples that have been stored several months or years or that have been fixed longer than needed for the used tissue size, extending the incubation at 90 °C from 40 minutes to 60 minutes can lead to a more efficient decrosslinking and result in higher yields. Incubation longer than 60 minutes at 90 °C is not recommended.

Excessive paraffin content

Large amounts of paraffin contained in a sample can make handling difficult and has a negative impact on yield and quality of DNA. For such samples, a reduction of the lysate volume and eluate volume is also to be expected. During sample preparation, as much surrounding paraffin as possible should be removed and only the “usable area” (shown in Figure 1) should be used for DNA extraction. This is especially recommended for DNA extraction of small FFPE tissue biopsies contained in a large piece of paraffin.

3. MATERIALS

3.1. Materials provided

Table 2: ***Content of EchoLUTION FFPE DNA Kit***

Product number	010-005-010	010-005-050	010-005-250
Product name	EchoLUTION FFPE DNA Kit (10)	EchoLUTION FFPE DNA Kit (50)	EchoLUTION FFPE DNA Kit (250)
Reactions	10	50	250
Lysis Buffer FFPE (LB)	1 × 1.1 mL	1 × 5.5 mL	1 × 27.5 mL
TurboLyse Protease FFPE (P)	1 × 100 µL	1 × 500 µL	1 × 2.5 mL
Removal Solution FFPE (RS)	1 × 2 mL	1 × 10 mL	2 × 25 mL
RNase FFPE (R)	1 × 10 µL	1 × 50 µL	1 × 250 µL
Low-TE Buffer (T)	1 × 1.5 mL	1 × 1.5 mL	2 × 1.5 mL
Spin Columns FFPE	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

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 and avoid the standing time of the spin columns mentioned in the protocol, we suggest using a swing-out rotor centrifuge with the 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 needs to reach a speed of 1,000 x *g*. When using a plate centrifuge, please use our Spin Column Adapter for Plate Centrifuges offered by BioEcho (product number 050-011-024).

B. Pipetting equipment

Pipetting can be performed using 10 µL and 200 µL single-channel pipettes.

C. Thermal shaker

The thermal shaker is used for the decrosslinking, tissue lysis, and removal of detergent and paraffin step. It needs to reach up to 90 °C and 1,400 rpm (e.g., Eppendorf® ThermoMixer® C). It is recommended to use two thermal shakers for the protocol.

D. Vortex mixer

A vortex mixer is required for lysate mixture.

4. STORAGE AND STABILITY

4.1. Kit reagents

- The EchoLUTION FFPE DNA Kit is shipped at ambient temperature.
- Upon kit arrival, TurboLyse Protease FFPE and RNase FFPE should be stored at 2–8 °C. The other kit components can be stored at 2–8 °C, but are also stable at room temperature (15–25 °C).
- If Removal Solution FFPE is stored at 2–8 °C, it may become solid. Please leave at room temperature till it becomes liquid again (30–90 minutes). If necessary, heat up to 56 °C to speed up the process.
- If Lysis Buffer FFPE is stored at 2–8 °C, a precipitate might form. Please leave the buffer at room temperature for a few minutes till the white precipitate is dissolved. If necessary, heat up to 56 °C for a few minutes to fully dissolve the precipitate.

4.2. Storage and stability of purified nucleic acids

For long-term storage of purified DNA, it is recommended to store the DNA samples at –20 °C. Avoid repeated thaw and freezing cycles; aliquot DNA if needed.

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.

Table 5: ***EchoLUTION FFPE DNA Kit safety information***

Component	Hazardous component	GHS symbol	Hazard statements	Precautionary statements	Additional statement
Removal Solution FFPE	112-30-1 Decanol	 GHS07 **Warning**	H319, H412	P101; P102; P103; P264; P273; P280; P305+P351+P338; P337+P313; P501	—
TurboLyse Protease FFPE	Subtilisin	 GHS06 GHS07 GHS08 GHS09 **Danger**	H315; H318; H334; H335; H400; H411	P101; P102; P103; P261; P284; P305+P351+P338; P310; P405; P501	—

Hazard Statements

H315: Causes skin irritation.
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.
H400: Very toxic to aquatic life.
H411: Toxic to aquatic life with long lasting effects.
H412: Harmful to aquatic life with long lasting effects.

Precautionary statements

P101: If medical advice is needed, have product container or label 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.
P264: Wash thoroughly after handling.
P273: Avoid release to the environment.
P280: Wear eye protection/face protection.
P284: [In case of inadequate ventilation] wear respiratory protection.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310: Immediately call a POISON CENTER/doctor.

P337+P313: If eye irritation persists: Get medical advice/attention.
P405: Store locked up.
P501: Dispose of contents/container in accordance with local/regional/national/international regulations.

6. DISPOSAL

Please follow local regulations regarding collection and disposal of hazardous waste and contact your waste disposal company to obtain information about laboratory waste disposal (waste code number 16 05 06). For more information, please refer to the instructions supplied with the safety data sheet (SDS). Please **contact us** for the SDS.
Dispose all biological samples and all liquid waste generated during the purification procedure as biohazardous waste.

A. Removal Solution FFPE and TurboLyse Protease FFPE

Very toxic or harmful to aquatic organisms with long-term effects. Discharge into the environment must be avoided. Do not allow undiluted or large quantities of the product to reach groundwater, watercourse or sewage system. Empty bottles may retain some product residues. Therefore, ensure the dispose of contents/container under local/regional/national/international regulations.

B. Other components and Spin Columns

No special measures for disposal are necessary. Components that have been in contact with potentially infectious material should be autoclaved. Used components may retain some buffer residues, which should be disposed of by local/regional/national/international regulations.

7. PROTOCOL

This protocol has been developed to extract total DNA from FFPE tissues using the EchoLUTION FFPE DNA Kit.

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

Preparation before starting:

- If Removal Solution FFPE and Lysis Buffer FFPE have been stored at 2–8 °C, please leave them at room temperature until the Removal Solution FFPE becomes liquid (up to 90 minutes) and no precipitate is visible in the Lysis Buffer FFPE (a few minutes). For details and further instructions please read section 4.1 Storage and Stability.
- Vortex EchoLUTION Spin Columns thoroughly to homogenize the 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 until step 4.

  NOTE: *For improved sedimentation of the matrix, we recommend that this step is performed upon receipt of the kit. Store them in upright position.*
- Heat the thermal shaker to 90 °C; if available, heat a second thermal shaker to 60 °C.
- Set the microcentrifuge to 1,000 × *g*.

  IMPORTANT NOTE: *Choose the × g (rcf) setting, not rpm.*
- Prepare 1.5 mL microcentrifuge tubes (not provided, preferably safe lock) for each sample.

1. FFPE tissue transfer

- When using FFPE blocks, cut sections using a microtome. Use of FFPE sections on slides is also possible. For both FFPE blocks and FFPE sections avoid transferring surrounding paraffin (Figure 1). For each sample, use 1–15 mg FFPE tissue or a minimum of 100 mm^2 and not more than 800 mm^2 of sections up to 10 µm thickness. Transfer the FFPE tissue into a 1.5 mL microcentrifuge tube.

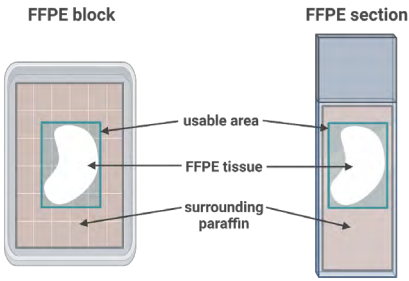


Figure 1: ***Schematic representation of an FFPE block and an FFPE section. Light brown: areas with paraffin only; white: area with fixed tissue; light green: area to be used for FFPE tissue extraction***

NOTE: *An excessive amount of surrounding paraffin can negatively influence the purification process resulting in reduced yield. Therefore, remove as much surrounding paraffin as possible. Genomic DNA yield is influenced by the FFPE tissue type and storage time. To ensure adequate yield from different FFPE tissues, adjust the amount of starting material accordingly.*
NOTE: *Sections thicker than 10 µm can result in lower yields due to inefficient removal of paraffin.*

2. *FFPE decrosslinking*

- Add 110 µL Lysis Buffer (LB) FFPE to each sample.
- Incubate samples at 90 °C for 40 minutes in a thermal shaker with constant shaking at 1,400 rpm and ensure that the tissue pieces are at the bottom.
  NOTE: *The degree of crosslinking in FFPE tissue depends on how long the fresh tissue was fixed in formaldehyde. For samples with a high degree of crosslinking, extending the incubation to 60 minutes at 90 °C can lead to more efficient decrosslinking resulting in higher yields. However, extending the incubation time can lead to a greater degree of DNA fragmentation.*
- Remove tubes from thermal shaker and let them cool down to room temperature (approximately 5 minutes).
  IMPORTANT NOTE: *Do not cool the tubes on ice.*
  NOTE: *If a second thermal shaker is not available, set the thermal shaker to 60 °C for step 3.*
  OPTIONAL: *Briefly centrifuge at low speed (e.g., 10 seconds at 1,000 × g) to collect the drops from the lid.*

3. *FFPE tissue lysis and removal of detergent & paraffin*

- Add 10 µL TurboLyse Protease (P) FFPE directly into the sample.
- Add 200 µL of Removal Solution (RS) FFPE to the reaction mix.
  IMPORTANT NOTE: *No master mix possible, because the two reagents do not mix.*
- Incubate at 60 °C for 30 minutes in a thermal shaker with constant shaking at 1,400 rpm for tissue lysis. Proceed with step 4 during incubation.
  NOTE: *If no second thermal shaker is available, leave the sample at room temperature until the temperature reached 90 °C.*
- Incubate at 90 °C for 5 minutes in thermal shaker with constant shaking at 1,400 rpm for protease inactivation.
  NOTE: *Extending the incubation time at 90 °C to 10 minutes can improve results depending on the tissue and age of the sample. However, extending the incubation time can also lead to more fragmentation of DNA.*

4. *Spin Column preparation & conditioning*

- Loosen the cap of the Spin Column by 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 × *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 Puncher correctly, punch a hole into 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 × g, discard flow-through and place the Spin Column in a fresh 1.5 mL reaction tube.*

5. DNA purification

- After incubation, centrifuge the samples (from step 3) for 2 minutes at maximum speed (minimum 10,000 × *g*) to separate the three phases (lower phase: aqueous, interphase: paraffin, upper phase: hydrophobic).

Optional for RNase digestion:

- Transfer the lower aqueous phase (lysate containing the DNA) into a new 1.5 mL microcentrifuge tube.

  NOTE: *Small amounts of the interphase containing paraffin or the hydrophobic upper phase have no negative effect on RNase digestion.*
- Add 1 µL RNase (R) to the lysate and homogenize by inverting several times or by vortexing the sample briefly.
- Incubate 2 minutes at room temperature.

- Transfer 80 µL from the lower aqueous phase (lysate containing the DNA) onto the prepared Spin Column.

  IMPORTANT NOTE: *Pipet slowly, drop-by-drop, and vertically onto the middle of the Spin Columns to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading!*

  NOTE: *If you have used the Cap Puncher make sure that you pipette vertically through the hole in the lid. Do not punch the pipette tip into the matrix while loading the lysate onto the EchoLUTION Spin Column.*
- If you did not use the Cap Puncher, close the cap of the Spin Column and loosen the cap again by half a turn.
- Centrifuge the loaded column for 1 minute at 1,000 × *g*.
- Purified DNA is in the flow-through.

The extracted DNA can be used immediately or stored. For long-term storage, place your DNA samples at –20 °C.

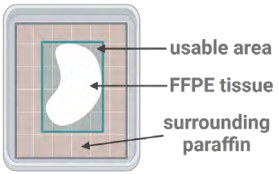
IMPORTANT NOTE: For spectrophotometric analysis, use the Low-TE Buffer (T) supplied with the kit as blank.

7.1. Quick protocol EchoLUTION™ FFPE DNA Kit

IMPORTANT NOTE: *Please use the quick protocol only after you have read and understood the complete user manual. Read the* ***preparation before starting*** *section of chapter 7 (Procotol).*

1. FFPE tissue transfer

- Transfer 100–800 mm² (thickness 10 µm) or 1–15 mg FFPE tissue from usable area into a 1.5 mL tube.



2. FFPE decrosslinking

- Add 110 µL LB.
- Incubate at 90 °C for 40 min at 1,400 rpm.
- Let cool down to RT (~5 min).

3. FFPE tissue lysis, detergent and paraffin removal

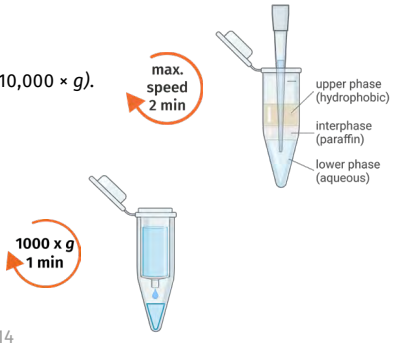
- Add 10 µL P directly into the sample.
- Add 200 µL RS.
- Incubate at 60 °C for 30 min at 1,400 rpm.
- Incubate at 90 °C for 5 min at 1,400 rpm.

4. Spin Column preparation & conditioning

- Loosen the cap of the Spin Column half a turn and snap off the bottom.
- Place Spin Column in a 2 mL reaction tube.
- Centrifuge 1 min at 1,000 × *g*.
- Discard flow-through.
- Place Spin Column in a new 1.5 mL reaction tube

5. DNA purification

- Tubes from step 3: Centrifuge 2 min at full speed (minimum 10,000 × *g*).
- Optional: Perform RNase digestion.
- Transfer 80 µL from lower aqueous phase (lysate) onto prepared Spin Column. Pipet slowly, drop-by-drop onto the middle of the column without touching the matrix.
- Centrifuge 1 min at 1,000 × *g*.
- Purified DNA is in the flow-through and ready-to-use.



8. QUALITY CONTROL

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

9. TROUBLESHOOTING

Observation	Comments and suggestions
DNA yield and concentration is low or **Poor performance in downstream experiments**	**Low DNA content of the tissue used** Some tissue samples have very low DNA yield. Use a reference sample or another extraction method to evaluate the results in comparison. **Small tissue samples used** To increase the nucleic acid concentration, it is possible to reduce the amount of Lysis Buffer FFPE from 110 µL to a minimum of 70 µL. This is useful for samples with a small total surface area when it is not possible to use several 10 µm sections. However, the reduction may result in more difficult handling and a lower yield, especially if the FFPE sample contains a lot of paraffin. When reducing the volume of the Lysis Buffer FFPE, at least 50 µL must be applied to the Spin Column in step 5. Please note that the volume reduction does not necessarily lead to a proportional increase of the nucleic acid concentration in relation to the reduced volume. **Insufficient preservation** The time that passes after a biopsy is excised and when it is embedded in paraffin influences DNA yield. Delaying preservation can lead to degradation. The larger the biopsy, the more inhomogeneous the tissue preservation is likely to be and results in lower yields. **Sample input** Always use a sufficient amount of sample to ensure appropriate experimental conditions. Use 1–15 mg FFPE tissue or a minimum of 100 mm^2 and not more than 800 mm^2 of sections up to 10 µm thickness. **Sample storage** The older a sample is, the less DNA can be extracted from

DNA yield and concentration is low

or

Poor performance in downstream experiments

(continuation)

it. For long-term storage, FFPE samples should be kept at 4 °C or –20 °C. FFPE sections on slides are exposed to air, which has a negative influence on the DNA.

Centrifuge settings
Most centrifuges offer the choice between rpm and *g*-force (rcf). To calculate the rpm, see chapter 2.2. Always make sure to stick to the correct time mentioned in the protocol to avoid insufficient elution of DNA.

Loading of the Spin Column
The correct loading of the Spin Column is crucial for the experimental outcome. 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.

Large amounts of paraffin
Too much paraffin in the sample can lead to decreased DNA yields and performance as well as a decrease in lysate volume. During sample preparation, as much surrounding paraffin as possible should be removed and only the "usable area" shown in Figure 1 should be used for DNA extraction. If your sample contained too much paraffin, you may not see a large amount of paraffin until the sample cools down to room temperature after phase separation and optional RNase digestion. In this case, heat up the sample again to 60 °C about 60 seconds until the paraffin is visibly melted. The paraffin melts again and it is easier for you to transfer the aqueous (lower) phase leaving most paraffin behind. Be aware that the risk of further fragmentation of the nucleic acids increases when the sample is heated again. It is best to work as fast as possible, so that additional heating is not required. See section 2.2 for further explanation.

Highly tilted matrix in column
A highly tilted matrix after conditioning can lead to insufficient time of interaction with the matrix, which can result in a poor extraction performance. However, when using centrifuges with a fixed angle rotor, the matrix does not become completely horizontal after conditioning. A slightly tilted surface according to the angle of the rotor (usually 30°) is to be

DNA yield and concentration is low or **Poor performance in downstream experiments** (continuation)	expected. This does not limit the purification ability. But if you observe that the matrix is tilted to a higher degree, we recommend prolonging the standing time to 30 minutes before conditioning. For quicker processing, we recommend vortexing the Spin Columns upon receipt of the kit and storing them in 2 mL microcentrifuge tubes in upright position till used. Alternatively, we suggest using a swing-out rotor centrifuge with our Spin Column Adapter for Plate Centrifuges (product number 050-011-024). **Insufficient decrosslinking** The degree of crosslinking of FFPE tissue depends on how long the fresh tissue was fixed in formaldehyde. For samples with a high degree of crosslinking or for very old samples, extending the 90 °C decrosslinking incubation to 60 minutes can lead to more efficient decrosslinking and thus result in higher yields. However, extending the incubation time can lead to a higher degree of DNA fragmentation.
A_{260}/A_{280} and/or A_{260}/A_{230} value is low	**Wrong blank in measurements** Use supplied Low-TE Buffer (T) as blank. **FFPE sample conditions** Please consider that FFPE samples are highly processed tissues often resulting in low A_{260}/A_{280} and/or A_{260}/A_{230} values. Use a reference sample or another extraction method to evaluate the results in comparison.
Degraded or fragmented DNA	**FFPE sample conditions** Fragmentation is common for FFPE tissues and can be caused by several reasons like exposure of fresh sample to oxygen, size of the tissue, fixation duration, storage conditions, storage time and more. On top also the decrosslinking of FFPE samples results in higher fragmentation of DNA compared to DNA from fresh samples. This is completely normal. Use a reference sample or another extraction method to evaluate the results in comparison. See section 2.2. for further information on handling FFPE tissues.

Degraded or fragmented DNA (continuation)	**Prolonged decrosslinking** For samples with a high degree of crosslinking or for very old samples, extending the 90 °C decrosslinking incubation to 60 minutes can lead to more efficient decrosslinking and thus result in higher yields. However, extending the incubation time can lead to a higher degree of DNA fragmentation.
Tilted matrix in column	**Inappropriate handling of Spin Column** A highly tilted matrix after conditioning can lead to insufficient time of interaction with the matrix, which can result in a poor extraction performance. However, when using centrifuges with a fixed angle rotor, the matrix does not become completely horizontal after conditioning. A slightly tilted surface according to the angle of the rotor (usually 30°) is to be expected. This does not limit the purification ability. But if you observe that the matrix is tilted to a higher degree, we recommend prolonging the standing time before conditioning. For quicker processing, we recommend vortexing the Spin Columns upon receipt of the kit and storing them in 2 mL microcentrifuge tubes in upright position till used. Alternatively, we suggest using a swing-out rotor centrifuge with our Spin Column Adapter for Plate Centrifuges (product number 050-011-024).
Occurrence of cross-contamination	**Contaminated pipettes** The use of contaminated pipettes can lead to cross-contaminations. We recommend a separate set of pipettes for sample preparation and PCR preparation, which should be cleaned thoroughly at regular intervals. It is also recommended to use filter tips for all pipetting steps involving samples. **Handling of samples** In general, always work cautiously when handling biological samples. Pay attention to what you're doing and always wear gloves. Keep tubes closed when possible. The use of pipette tips with filters is recommended.

Eluate is missing or volume too low	**Inappropriate handling of Spin Column** The Spin Column was closed during centrifugations, and vacuum was generated. See chapter 2.2 for further instructions on how to handle Spin Columns. **Centrifuge settings** Most centrifuges offer the choice between rpm and *g*-force (rcf); if not, calculate the rpm as described in chapter 2.2. Always make sure to use the correct time mentioned in the protocol to ensure sufficient elution of DNA. **Large amounts of paraffin** Too much paraffin in the sample can lead to a reduction of lysate and eluate volume. During sample preparation, remove as much surrounding paraffin as possible, and use only the "usable area" (shown in Figure 1). If your sample contains too much paraffin, you may not see a large amount of paraffin until the sample has cooled down to room temperature after decrosslinking. In this case, heat up the sample again to 60 °C for a maximum of 3 minutes until the paraffin is visibly melted. Be aware that the risk of further fragmentation of the nucleic acids increases when the sample is heated a second time. Once the paraffin is melted, it is easier to transfer the aqueous (lower) phase, leaving most of the paraffin behind. If the time is not sufficient to melt the paraffin completely, you can centrifuge the sample for 4 minutes at full speed to achieve a more compact interphase containing the paraffin. If you are worried about further fragmentation, you can alternatively skip the heating step (60°C for maximum of 3 minutes) and only centrifuge the sample for 4 minutes at full speed to compress the interphase and make the lower phase more accessible for transfer. See chapter 2.2 for further explanation.

For questions and further troubleshooting, please contact us!

10. LIMITATIONS OF USE

Limitations regarding EchoLUTION FFPE DNA Kit are listed as follows:

- Strict compliance with the user manual is required for successful 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 co-purification of impurities.
- The DNA yield varies and is dependent on several factors, including the technique used to take the sample and the type of FFPE preparation.
- The proof of principle for the EchoLUTION FFPE DNA Kit was evaluated and confirmed using state-of-the-art RT-qPCR and DNA sequencing. Performance parameters are highly dependent on the quality of sample collection.
- This kit is for research use only.

11. SYMBOLS

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

Table 6: ***EchoLUTION FFPE DNA 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 respective product page.

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

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