EchoLUTION™ Tissue RNA Kit

Spin column kits

USER MANUAL

RUO

REF

011-015-050

011-015-250

BioEcho Life Sciences GmbH | BioCampus Cologne, Main Building | Nattermannallee 1 | 50829 Cologne, Germany

TABLE OF CONTENTS

1. INTENDED PURPOSE

The BioEcho EchoLUTION Tissue RNA Kit is intended for easy, rapid, and efficient RNA extraction from mammalian tissue samples (liver, muscle, spleen, lung, and nervous tissues). The excellent yield and purity of total RNA obtained with the EchoLUTION Tissue RNA Kit allows use in downstream applications without further processing.

The EchoLUTION Tissue RNA Kit is intended for research use only.

2. EXPLANATION OF THE KIT

The EchoLUTION Tissue RNA Kit is characterized by the EchoLUTION single-step purification technology and tailored lysis. Together they reduce the overall extraction time and workflow to a minimum with consistent results and sensitivity compared to state-of-the-art methods.

The EchoLUTION Tissue RNA Kit benefits are:

- Short processing time
- Ultra-fast lysis
- Few protocol steps
- High sample throughput with minor equipment and capital investment
- Up to 54 % less plastic waste compared to conventional methods
- No toxic reagents

For further details about kit specifications, see Table 1.

Table 1: ***Kit specifications***

Specification	Description
Sample input	Up to 20 mg of mammalian tissue depending on tissue type
Sample type	Liver, muscle, spleen, lung, and nervous tissue
Sample condition	Fresh-frozen or stabilized
Purified nucleic acid	Total RNA including small RNA
Elution volume	Up to 80 µL
Expected yields	Up to 14 µg depending on tissue type

2.1. *Single-step purification principle*

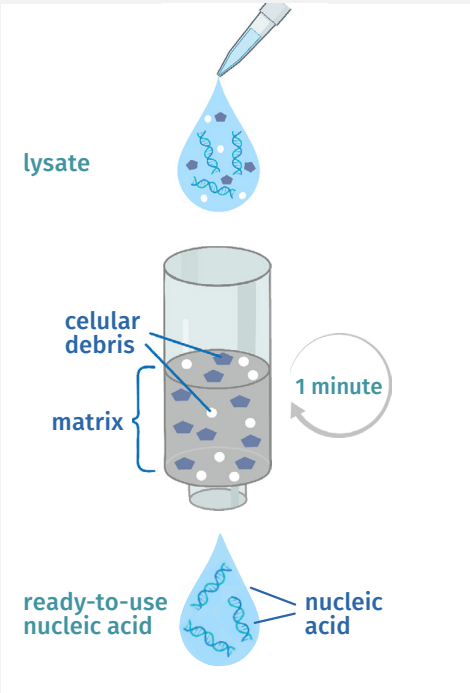
All BioEcho nucleic acid extraction kits are based on the EchoLUTION technology, which consists of tailored sample processing that includes lysis followed by single-step purification. The purification step works differently than conventional methods such as magnetic-bead and silica kits based on the 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 the 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 absorption. 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. 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 Spin Columns, be sure to use the recommended *g*-force centrifuge settings. Most centrifuges offer the choice between rpm and *g*-force (rcf); if not, 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 RNA

For high-quality RNA extraction, the samples must be free of ribonucleases (RNases), as these enzymes digest RNA very efficiently. In general, RNases are very active, stable, and difficult to inactivate. Even small amounts can digest sufficient RNA to drastically reduce the yield. To avoid RNase contamination of your samples, some things need to be considered. Always eliminate possible RNase contamination from any consumables you will use before starting your extractions. Make sure that the working area is free of RNases throughout the whole procedure to avoid introducing RNases into the samples.

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

In general, aseptic techniques should always be used when working with RNA to avoid contamination risk. The most common sources of RNases are dust and hands, as they can hold bacteria and molds. Therefore, always wear gloves while handling reagents and RNA samples. Replace gloves regularly and keep tubes closed when possible.

For long-term storage, purified RNA can be stored at –80 °C. Samples can be aliquoted to avoid repeated freeze-thaw cycles. When working with the RNA for downstream applications, thaw on ice and keep on ice while working. If you do not freeze your RNA right-away, put it on ice immediately after finishing the purification protocol!

Input material

For optimal results, it is vital to use the correct amount of input material to achieve best RNA purity and yield.
Factors that influence the RNA yield:

- Different tissue types have different RNA and DNA content. Using more than the recommended amounts per sample (max. 10 mg tissue) could lead to handling difficulties and inhibition in downstream experiments.
- Incorrect volume of lysis buffer and insufficient lysis lead to low RNA recovery.
- Extended lysis time leads to degradation of the RNA.

Yields to be expected

Table 2: ***Total RNA yield examples; extractions performed from rodent tissues***

Classification	Tissue samples (input)	RNA yield [µg]
High-yield tissue	Spleen (5 mg)	10.4
	Liver (5 mg)	14
Low-yield tissue	Cortex (10 mg)	8.4
	Muscle (10 mg)	8
	Lung (10 mg)	10
	Brain (10 mg)	6.7

Lysis and homogenization of tissue material

For efficient mechanical disruption, we highly recommend bead-beating. The mechanical disruption of the tissue material is the most relevant parameter determining RNA yield.

Homogenization of the sample is also important to reduce the viscosity of the lysates, to ensure precise handling and pipetting. Cell clumps remaining in the lysate can lead to reduced RNA yield and handling issues.

Correct lysis of the tissue material is necessary to release the RNA from the tissue. Incomplete lysis results in drastically reduced RNA yields.

For the lysis step, it is important to stick to the time and temperature given in the protocol, as longer lysis time can lead to degradation of the RNA and contamination with genomic DNA.

Homogenizer methods:

- Mixer Mills (Retsch): Place tissue material (frozen or stabilized) with a stainless-steel bead (4 mm) and ensure the vessel is closed. Depending on tissue type, homogenize samples 4 x 30 seconds at 30 Hz (1,800 rpm) or until no more chunks are visible.
- FastPrep® Instruments (MP Biomedicals®) or Precellys® Evolution Touch (Bertin Technologies): Homogenization time and speed might vary depending on the tissue types and instrument. You can try the predefined protocols and optimize depending on your tissue sample. Please refer to the equipment manufacturer's recommendations for further protocol setups and instructions.

3. MATERIALS

3.1. Materials provided

The kit contains a lysis buffer and a lysis solution that require preparation before they can be used for the first time. Please read section 4.1 before starting your RNA extraction.

Table 3: ***Content of EchoLUTION Tissue RNA Kit, spin column format***

Product number	011-015-050	011-015-250
Product name	**EchoLUTION Tissue RNA Kit (50)**	**EchoLUTION Tissue RNA Kit (250)**
Reactions	50	250
Lysis Buffer Tissue RNA concentrate* (LB)	112.5 mg	2 × 270 mg
Lysis Solution Tissue RNA* (LS)	7.5 mL	2 × 19.5 mL
Clearing Solution Tissue RNA (CS)	1.2 mL	6 mL
DNase Tissue (D)	260 µL	2 × 650 µL
Reaction Buffer DNase Tissue (RB)	110 µL	550 µL
Low-TE Buffer (T)	1.2 mL	1.2 mL
Spin Columns Tissue RNA	50	250

** For correct preparation of these components, read section 4.1.*

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.

B. BioEcho Steel Beads

For fast and efficient tissue disruption and sample homogenization, we recommend using stainless-steel beads of 4 mm. BioEcho offers a suitable product. To purchase this item, use the product numbers 050-006-002 and 050-006-010, depending on the number of beads required to process your samples. Alternatively, use our convenient Homogenization Set (see Table 4, product numbers 030-006-050 and 030-006-250).

C. Micro Tubes

These tubes are required for the preparation of master mixes and performing the DNA removal.

D. Lysis Tubes

The Lysis Tubes necessary for mixing the Lysis Buffer with the tissue sample are not included in the kit. We recommend using our Homogenization Set (product numbers 030-006-050 and 030-006-250). However, you can also use any lysis tube. For bead beating the lysis tube must be tightly closed.

Table 4: ***Content of Homogenization Set, spin column format***

Product number	030-006-050	030-006-250
Product name	**Homogenization Set (50)**	**Homogenization Set (250)**
Reactions	50	250
Lysis Tubes with Steel Bead, 4 mm	50	250

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, we suggest using a swing-out rotor centrifuge with our Spin Column Adapter for Plate Centrifuges (product number 050-011-024).

Activities that are likely to involve a bioaerosol hazard must be performed in a safety microbiological cabinet (MSC) or a comparable facility for personal protection (e.g., fume high-efficiency particulate air filter).

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 at least 13,000 x *g*. When using a plate centrifuge, please use our Spin Column Adapter for Plate Centrifuges (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 is used for the lysis step. It needs to reach up to 40 °C and 1,400 rpm (e.g., Eppendorf® ThermoMixer® C). Alternatively, you can use a heating block or heat chamber.

D. Vortex mixer

A vortex mixer is recommended for reagents and lysate mixture.

E. Homogenizer

Required for tissue disruption and sample homogenization. Suitable for single tubes (see section 2.2) and with a minimum power of 30 Hz.

F. Ceramic blade scalpel

BioEcho offers a scalpel suitable for cutting tissue material. This product can be ordered under the product number 050-002-001.

4. STORAGE AND STABILITY

4.1. Kit and reagents

- The EchoLUTION Tissue RNA Kit is shipped at ambient temperature. The DNase is shipped cooled in a separate box.
- Upon kit arrival, the Lysis Buffer Tissue RNA (LB) should be stored at 2–8 °C. The DNase should be stored in a freezer (-20°C). The other kit components are stable at room temperature (15–25 °C).

Before starting RNA extraction with the **EchoLUTION Tissue RNA Kit (REF: 011-015-050 and REF: 011-015-250)**, prepare the following:

- Add the complete Lysis Solution Tissue RNA (LS) to the Lysis Buffer Tissue RNA (LB) (brown bottle). Mix by inverting 5–10 times, then let stand for 10 minutes to reduce the foam. After mixing, indicate the addition of the Lysis Solution Tissue RNA (LS) and the date. Prepared Lysis Buffer Tissue RNA (LB) will be stable for six months when stored at –20 °C. If storage is not possible at this temperature, the Lysis Buffer Tissue RNA (LB) can be stored at 2–8 °C for two weeks (mark date on the bottle to make sure buffer is not expired).
- Lysis Solution Tissue RNA (LS) or prepared Lysis Buffer Tissue RNA (LB) may form precipitates upon storage below room temperature. Always check for precipitation before use and redissolve by allowing the buffer to warm up to room temperature for a minimum of 10 minutes or until precipitation is dissolved.

Table 5: ***Stability of EchoLUTION Tissue RNA Kit components***

Component	Stability
Lysis Buffer Tissue RNA concentrate (LB)	Unopened stable at 2–8 °C until expiration date mentioned on label; Prepared Lysis Buffer Tissue RNA stable at –20 °C for six months or at 2–8 °C for two weeks
Lysis Solution Tissue RNA (LS)	Stable at 15–25 °C until expiration date mentioned on label
Clearing Solution Tissue RNA (CS) **DNase Reaction Buffer (RB)** **Low-TE Buffer (T)** **Spin Columns**	Stable at 2–25 °C until expiration date mentioned on label
DNase Tissue (D, Box 2)	Stable at –20 °C until expiration date mentioned on label

4.2. Sample collection

Tissue samples can be collected and processed fresh frozen or stabilized in PurifyLater (BioEcho product number 030-002-100 or 030-002-500) or RNAlater® (Thermo Fisher Scientific®).

A. Handling frozen tissue for RNA extraction

Keep samples at –70/–80 °C until you are ready to begin.

If samples cannot be processed immediately, tissue chunks can be stored at –70 °C/–80 °C until use. Important is to avoid freeze–thaw cycles.

Before freezing, tissue mass should be determined to make sure that amount of tissue adjusted to protocol requirements.

4.3. Storage and stability of purified nucleic acids

For long-term storage of purified RNA, it is recommended storing the RNA samples at –80 °C. Avoid repeated thaw and freezing cycles, aliquot RNA 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 6: ***EchoLUTION Tissue RNA Kit safety information***

Component	Hazardous component	GHS symbol	Hazard statements	Precautionary statements	Additional statement
Clearing Solution (CS)	Strontium chloride	 Danger	H318	P101; P102; P103; P280; P305+P351+P338 P310; P501	—
Lysis Buffer Tissue RNA (LB)	Tris (2-carboxyethyl) phosphine hydrochloride (TCEP)	 Danger	H314; H318	P101; P102; P103; P260; P303+P361+P353; P305+P351+P338; P310; P405; P501	—

Hazard Statements

H314: Causes severe skin burns and eye damage.
H318: Causes serious eye damage.

Precautionary statements

P101: If medical advice is needed, have product container or label at hand.
P102: Keep out of reach of children.
P103: Read carefully and follow all instructions.
P260: Do not breath dusts or mists.
P280: Wear eye protection / face protection.
P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
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.
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 and 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

Components that have come into contact with potentially infectious material should be autoclaved. Used components may retain some buffer residues, which should be disposed of according to local/regional/national/international regulations.

The storage buffer of the Spin Columns collected in the collection tube during the conditioning step can be disposed of in the sink or on a paper tissue.

7. PROTOCOL

This protocol has been developed to extract total RNA from mammalian tissue samples (e.g., liver, muscle, spleen, lung, and nervous tissues) using the EchoLUTION Tissue RNA Kit. This is not a silica-based kit. Please read the instructions carefully before starting!

Preparation before starting:

- Vortex EchoLUTION Spin Column 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.

  NOTE:

  - *For improved sedimentation of the matrix, we recommend performing this step the day before and letting the columns stand overnight.*

- Pre-heat the thermal shaker to 40°C.
- Add the complete Lysis Solution RNA (LS) to the Lysis Buffer RNA (LB) (brown bottle). Mix by inverting 5–10 times, then let stand for 10 minutes to reduce the foam. After mixing, indicate the addition of the lysis solution and the date. Prepared Lysis Buffer RNA will be stable for six months when stored at –20 °C. If storage is not possible at this temperature, the Lysis Buffer RNA can be stored at 2–8 °C for two weeks (mark date on the bottle to make sure buffer is not expired).
- Prepared LB may form precipitate upon storage below room temperature. Before use redissolve by allowing it to warm up to room temperature for minimum 10 minutes or until precipitation is dissolved.
- If tissue samples are pre-loaded in Lysis Tube and stored at –70 °C/–80 °C, defrost for 2–5 minutes at 4°C. If the tube is too cold, the water-based lysis buffer will freeze and bead beating is not sufficient.
- Set the microcentrifuge to 1,000 x *g*.
- Prepare a DNase reaction mix for the number of samples with 10 % excess volume (according to table 7), mix gently and keep cool.
- Carry out the complete RNA extraction at room temperature.

  IMPORTANT NOTES:

  - *Choose x g (rcf), not rpm, unless stated otherwise.*
  - *Make sure the buffer LB is prepared and warmed up to room temperature.*

Table 7: ***DNase reaction mix, for one reaction***

Component	Volume
DNase	5.0 µL
Reaction Buffer	2.0 µL
Final volume	**7.0 µL**

1. *Spin Column preparation*

- 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 x *g*, and discard flow-through.
- Place the Spin Column in a fresh 1.5 mL reaction tube (not provided).
- Proceed directly with step 2.

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 x g, discard flow-through and place the Spin Column in a fresh 1.5 mL reaction tube.

2. *Homogenization and lysis*

- Transfer appropriate amount of tissue material and a 4 mm steel bead (not provided) into each Lysis Tube (not provided) while tube is cooled on dry ice to avoid RNA degradation during sample loading.

NOTES:

- *Recommended amount 5-10 mg according to table 2.*
- *For tough-to-lyse tissues more than one bead might be necessary.*
- *For efficient lysis and homogenization you can use the BioEcho Homogenization Set (product numbers 030-006-050 and 030-006-250).*

SAVE STOPPING POINT:

- *Lysis Tubes with tissue and bead (homogenized or non- homogenized) can be stored at –70 °C/–80 °C for later use. Make sure to defrost tube before adding LB to prevent freezing of LB.*

- Add 150 µL Lysis Buffer Tissue RNA (LB) to each Lysis Tube and close tightly.
- Place the Lysis Tube in the homogenizer for sample disruption. See section 2.2 for further information on homogenization.
- Homogenize samples in bead beater for 4 × 30 s at 30 Hz.

NOTE:

- *This setting will be sufficient for most types of tissue, but this step may have to be optimized according to your sample texture.*

- Centrifuge tubes for 30 sec at maximum speed (up to 20,000 × *g*) to reduce the foam.
- Incubate lysate at 40 °C in a thermal shaker with constant shaking of 1,400 rpm for 5 minutes.

NOTES:

- *Longer lysis time can lead to degradation of the RNA.*
- *An additional centrifugation step after lysis may be required to reduce the foam.*

- After lysis is finished, add 22 µL Clearing Solution (CS) to the lysates and mix thoroughly.

- Centrifuge tubes for 2 minutes at maximum speed (up to 20,000 × *g*) to pellet debris at the bottom of each tube.

NOTE:

- *If you are using a plate centrifuge with Spin Column Adapter, prolong the centrifutation to 5 minutes.*

3. gDNA removal

- Carefully transfer up to 100 µL supernatant to a clean microtube (not provided).
- Add 7 µL of the DNase reaction mix (see table 7) directly to the lysates and mix gently.

OPTIONAL:

- *Dispense DNase reaction mix into the microtubes before adding the supernatant. Cool at 4 °C before use.*

- Incubate 10 minutes at room temperature.

4. RNA purification

- After gDNA removal is finished, open the Spin Column and slowly transfer up to 80 µL of the lysate to the Spin column.

IMPORTANT NOTES:

- *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!*
- *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.*

- 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 extracted RNA can be stored at -80°C or put on ice and used directly.

IMPORTANT NOTE:

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

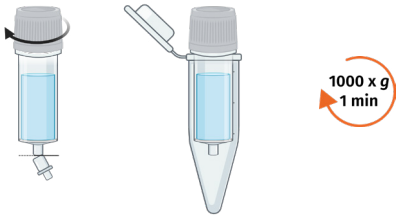
7.1. Quick protocol EchoLUTION™ Tissue RNA Kit: spin column kits

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

Preparation before starting

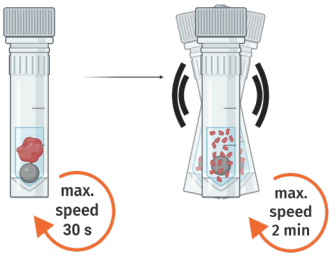
- Prepare the Lysis Buffer Tissue RNA (LB).
- Pre-heat the thermal shaker to 40 °C.
- Set the microcentrifuge to 1,000 x *g*.
- Prepare a DNase reaction mix.

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

2. Homogenization and lysis

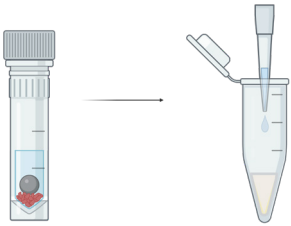


- Place one tissue sample in each Lysis Tube with steel bead.
- Add 150 µL LB.
- Close tube tightly.
- Bead beat 4 x 30 sec at 30 Hz
- Centrifuge 30 sec max. speed
- Incubate at 40 °C for 5 min with constant shaking at 1,400 rpm.
- Add 22 µL Clearing Solution and mix.
- Centrifuge 2 min at max. speed (up to 20,000 x *g*).

IMPORTANT NOTE:

Longer incubation can lead to RNA degradation. Therefore, it is highly important to not prolong the incubation period.

3. gDNA removal



- Transfer 100 µL of cleared lysate to a new microtube.
- Add 7 µL DNase reaction mix and mix gently.
- Incubate for 10 min at room temperature.

4. RNA purification

- Transfer up to 80 µL lysate. Pipet slowly, drop-by-drop onto the middle of the column without touching the matrix.
- Centrifuge spin column for 1 min at 1,000 x *g*.
- Purified RNA is in the flow-through and ready-to-use. Freeze at –80 °C or put on ice immediately for further use.

8. QUALITY CONTROL

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

To request the Certificate of Analysis (CoA), please contact us.

9. TROUBLESHOOTING

Observation	Comments and suggestions
RNA yield and concentration is low	**The tissue type used has low RNA content** Some tissues have very low RNA yield. Also, the storage conditions can lead to variations in RNA content. **Sample input** Use the correct amount of tissue input to ensure appropriate experimental conditions. Depending on the tissue type, the optimal amount may vary. If the tissue amount described in the protocol is not working or you want to extract RNA from a type of tissue you have not worked with, you might need to run an assessment to determine the optimal input material required for the extraction. For questions about complicated-to-extract or new tissue samples, please contact us. **Incomplete sample disruption** Incorrect lysis and homogenization of the starting material results in drastically reduced RNA yields. If lysis is incomplete, the tissue does not release all the RNA contained in the sample. Make sure to use the correct lysis buffer volumes. Insufficient homogenization of the sample leads to enhanced viscosity of the lysates and thereby to handling issues. Remaining tissue clumps can lead to homogenize release of RNA. Make sure to always homogenize the sample thoroughly, as stated in the protocol, and check visually.

RNA yield and concentration is low (continuation)	**Incorrect lysis conditions** In the lysis step it is important to stick to time and temperature mentioned in the protocol, as longer lysis time can lead to degradation of the RNA. Make sure that lysis buffer covers the complete tissue, to ensure lysis. **Loading of purification matrix** The correct loading of the Spin 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. **Centrifuge settings** Most centrifuges offer the choice between rpm, and *g*-force (rcf); if 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. **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. Please read observation "Highly tilted matrix in column" for further instructions.
Degraded RNA	**Incorrect storage of tissue** Extension of storage time can lead to RNA degradation. Make sure to store tissue in RNA stabilization reagent according to manufacturer's recommendations. Frozen tissue should be stored at –70 °C/–80 °C until use. Important is to avoid freeze/thaw cycle without protection by stabilizers, see section 4.2. A. Handling frozen tissue for RNA extraction. **Contamination with RNase** RNases digest RNA very efficiently, even small amount can digest the RNA and lead to poor experimental outcome. Even though, included materials and

Degraded RNA (continuation)	solutions are RNase-free, RNases can be introduced while handling the samples. RNase contamination of your samples needs to be avoided. The working area and materials need to be RNase-free throughout the whole procedure, see section 2.2 for detailed instructions. We highly recommend using specific workplaces and equipment that have not been used in DNA preparations including RNase digests. Always keep extracted RNA cold and freeze at –80 °C as soon as possible. **Incorrect lysis conditions** In the lysis step it is important to use time and temperature mentioned in the protocol, as longer lysis time can lead to degradation of the RNA.
DNA contamination	**Too much sample input** Always use up to 10 mg per sample to ensure appropriate experimental conditions. If this amount of tissue is too much (due to high nucleic acid content), reduce the mass of tissue used. **Low RNA content of cells** Some tissues have very low RNA content, what can lead to inappropriate extraction conditions. **Incorrect lysis conditions** Longer lysis time and temperature can lead to contamination with genomic DNA. Use temperature and time mentioned in the protocol.
Poor performance in downstream experiments	**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. Please read observation “Highly tilted matrix in column” for further instructions.

Poor performance in downstream experiments (continuation)	**Centrifuge settings** Most centrifuges offer the choice between rpm and *g*-force (rcf); if 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. **Loading of purification matrix** The correct loading of the Spin 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.
Occurrence of cross-contamination	**Contaminated pipettes** The use of contaminated pipettes can lead to cross-contamination. BioEcho recommends using a separate set of pipettes for sample preparation and PCR preparation. The pipettes 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, work cautiously using sterile microbiological practices when working with RNA. To avoid risk of contamination, always wear gloves while handling reagents and RNA samples. Replace gloves regularly and keep tubes closed when possible. The use of pipette tips with filters is recommended.

Eluted RNA has a light brown color

Sample input

Depending on the type of tissue, the ideal sample input weight should be determined and maintained. A light brown color eluate may indicate overload of the matrix. We recommend reduction of the input sample material. Reducing the loading volume is also an option to improve the purity of the eluted RNA.

Pay attention to correct loading of the purification matrix.

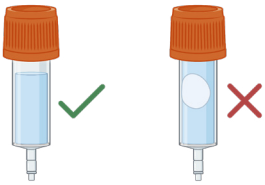
Air bubbles in Spin Column matrix

Inappropriate homogenization

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.

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.

For improved sedimentation of the matrix, we recommend that this step is performed upon receipt of the kit and store them in an upright position, but at least 10 minutes before preparation.

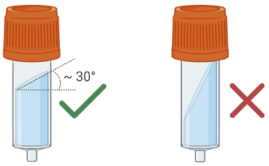


Highly tilted matrix in column

Inappropriate handling of Spin Column

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 up to overnight 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. See section 2.2 for further instructions.



Another reason could be that the Spin Column was completely closed during centrifugation and a vacuum was generated. 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).

A_{260}/A_{280} and A_{260}/A_{230} values are low	**Wrong blank in measurements** Use supplied Low-TE Buffer (T) as blank in measurements. **Incorrect lysate volume** Avoid overloading the purification matrix by increasing lysate volume. Using a higher volume than the one recommended in the protocol will compromise the sample purity. **Sample input too high** Always use appropriate amount per sample to ensure optimal experimental conditions (see table 2). If this amount of tissue is too much, repeat with lower mass.
Eluate is missing or volume to low	**Inappropriate handling of Spin Column** The Spin Column was closed during centrifugation, and a vacuum was generated. See section 2.2 for further instructions. **Centrifuge settings** 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.

For questions and further troubleshooting, please contact us!

10. LIMITATIONS OF USE

Limitations regarding EchoLUTION Tissue RNA Kit are listed below:

- Strict compliance with the user manual is required for RNA purification. Following good laboratory practices is crucial for the successful use of the product. Appropriate handling of the reagents is essential to avoid contamination and impurities.
- The proof of principle for the EchoLUTION insert product name Kit was evaluated and confirmed using state-of-the-art RT-qPCR and RNA sequencing. Performance parameters are highly dependent on the quality of sample collection.
- The kit is for research use only.

11. SYMBOLS

The following table describes the symbols that appear on the labeling of the EchoLUTION Tissue RNA Kit.

Table 8: ***EchoLUTION Tissue RNA Kit symbols***

Symbols	Description
	Manufacturer
	For research use only
	Product number
	Batch code
	Contains sufficient for < n > reactions
	Temperature limitation
	Do not reuse
	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!

+49 221 99 88 97-0

contact@bioecho.de

BioEcho Life Sciences GmbH
BioCampus Cologne, Main Building
Nattermannallee 1
50829 Köln/Cologne, Germany

BioEcho Life Sciences, Inc.
400 Tradecenter Suite 6900
Woburn, MA 01801, USA

www.bioecho.com