EchoLUTION™ Tissue RNA Kit

96-well plate kits

USER MANUAL

RUO

REF

011-115-002

011-115-008

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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	High RNA content (e.g., spleen, liver): 5 mg Low RNA content (e.g., muscle, lung, nervous tissues): 10 mg
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 (see table 2)

2.1. *Single-step purification principle*

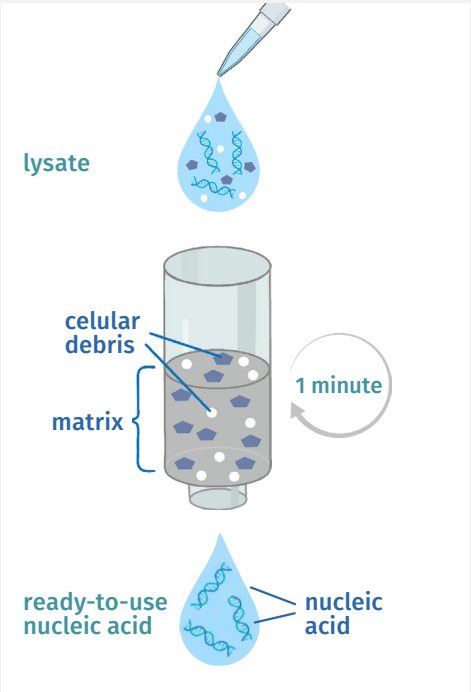
All BioEcho nucleic acid extraction kits are based on the EchoLUTION technology, which consists of tailored sample processing that includes lysis followed by single-step purification. The purification step works differently than conventional methods such as magnetic-bead and silica kits based on the 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 adsorption. 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 within the Purification Plate 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 Purification Plate, 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 thaw and freeze 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 × 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.

Automation

The EchoLUTION Tissue RNA Kit has been demonstrated on the Hamilton® Microlab® STAR™ liquid handling platform to purify RNA for 96-well formats in a fully, or semi-automated system, as well as on the SPT Labtech apricot S3 (semi-automated). Other liquid handling platforms can be used for RNA extraction. We will be happy to work together to develop tailored automated protocols that fit your needs. Please, contact us to get more information.

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, 96-well plate format***

Product number	011-115-002	011-115-002
Product name	**EchoLUTION Tissue RNA Kit (2 × 96)**	**EchoLUTION Tissue RNA Kit (8 × 96)**
Reactions	192	768
Lysis Buffer Tissue RNA concentrate* (LB)	412.5 mg	4 × 412.5 mg
Lysis Solution Tissue RNA* (LS)	30 mL	4 × 30 mL
Clearing Solution Tissue RNA (CS)	4.35 mL	18 mL
DNase Tissue (D)	1.1 mL	4 × 1.1 mL
Reaction Buffer DNase Tissue (RB)	500 µL	4 × 500 µL
Low-TE Buffer (T)	1.2 mL	2 × 1.2 mL
Purification Plate 96 Type 4	2 plates	8 plates
Elution Plate 96 Type 1	2 plates	8 plates
Adhesive Foil	2 foils	8 foils

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

3.2. Materials required but not provided

A. Conditioning Plate

The Conditioning Plate is necessary to remove the matrix storage buffer from the Purification Plate. The Conditioning Plate can be reused up to 20 times. Please remove the collected buffer after every use. Please do not use any alternative plate. The plate needs to be ordered separately. To purchase this item, use the product number 060-001-002 or 060-001-008, depending on the number of plates required.

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 030-006-102 and 030-006-108).

C. Tubes for master mixes

These tubes are required for the preparation of master mixes.

D. Multichannel reagent reservoir

These reservoirs are necessary when using multichannel pipettes for transferring prepared master mixes or buffers.

E. Lysis Plate

The lysis plate necessary for mixing the Lysis Buffer with the tissue sample is not included in the kit. We recommend using BioEcho Lysis Plate Type 1 as part of our Homogenization Set (product numbers 030-006-102 and 030-006-108). However, you can also use any Lysis Plate. In this case, the lysis plate should be a 96-well plate with a capacity of at least 500 µL per well. For bead beating the lysis plate must be tightly closed. When using the above-mentioned BioEcho Lysis Plate we offer suitable Cap Strips as part of our Homogenization Set.

F. Micro Plate for DNA removal

The micro plate necessary for performing the DNA removal is not included in the kit. We offer a suitable Micro Plate for DNA Removal as part of our Homogenization Set (product numbers 030-006-102 and 030-006-108). However, you can also use any micro plate.

G. Plates for counterbalance in centrifuge

In case an odd number of plates is processed, prepare an additional plate stack to ensure the centrifuge is balanced and fill the wells with the appropriate amount of water.

Table 4: ***Content of Homogenization Set, 96-well plate format***

Product number	030-006-102	030-006-108
Product name	**Homogenization Set (2 × 96)**	**Homogenization Set (8 × 96)**
Reactions	192	768
Lysis Plate Type 1	2 plates	8 plates
Cap Strips	2 packs of 12	8 packs of 12
Steel Beads, 4 mm	200 beads	2 × 400 beads
Micro Plate 96	2 plates	8 plates

3.3. Laboratory equipment needed

A. Plate centrifuge

For the procedure, plate centrifuges with the following specifications are mandatory:

- Standardized Society for Biomolecular Screening (SBS) format
- Capable of at least 4,500 x *g*.
- Capable of holding plate stacks of 5 cm height
- Swing-out rotor

B. Pipetting equipment

Pipetting can be performed using a single-channel pipette as well as a multi-channel pipette for pipette. We recommend using wide-bore tips for mixing and transferring the lysate to the purification matrix.

C. Thermal shaker for plates

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 with SmartBlock DWP 1000). Alternatively, you can use a heating block or heat chamber.

D. Homogenizer

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

E. 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 Purification Plates and 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).
- The Purification Plates need to be stored as described on the label (label facing up).

Before starting RNA extraction with the **EchoLUTION Tissue RNA Kit (REF: 011-115-002 and REF: 011-115-008)**, 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 2 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)** **Elution Plates** **Lysis Plates**	Stable at 2–25 °C until expiration date mentioned on label
Purification Plates	Unopened stable at 2–8 °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.

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. When samples are stored frozen into Lysis Plate, the plate should be slightly thawed at room temperature before adding the lysis buffer in step 2, to avoid freezing of the lysis buffer.

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 Purification Plates

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

The storage buffer of the Purification Plates collected in the Conditioning Plate 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:

- 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 Plate and stored at –70/–80 °C, let plate defrost for 20–30 minutes at 4°C. If plate is too cold, the water-based Lysis Buffer will freeze and bead beating is not sufficient.
- Set centrifuge to 1,000 x *g*.
- Prepare a DNase reaction mix for the number of samples with at least 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***

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

1. *Purification Plate preparation*

- Detach first the lower and then the upper foil from the Purification Plate. Be sure to keep the plates in a horizontal position while removing the foils, as the wells contain liquid.
- Place the Purification Plate on top of the Conditioning Plate (not provided, product number 060-001-002 or 060-001-008).

- Centrifuge plate stack for 1 minute at 1,000 x *g*, discard flow-through.
- Place the Purification Plate on top of the Elution Plate.
- Proceed directly with step 2.

NOTES:

- *The centrifuge rotor should be capable of holding plate stacks that have a height of 5 cm.*
- *Conditioning Plates can be reused.*
- *If the Purification Plate was not shipped or stored upright, the matrix may stick to the upper foil. In this case, shake plate until the matrix is removed from upper foil.*
- *Make sure the foil is completely removed from the bottom.*

2. *Homogenization and lysis*

- Transfer appropriate amount of tissue material and a 4 mm steel bead (not provided) into each well of the Lysis Plate (not provided) while plate 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 030-006-102 and 030-006-108).*

SAVE STOPPING POINT:

- *Sealed plate with tissue and beads (homogenized or non-homogenized) can be stored at –80°C for later use. Make sure to defrost plate before adding LB to prevent freezing of LB.*

- Add 150 µL Lysis Buffer Tissue RNA (LB) to each well with tissue and close the Lysis Plate tightly.
- Place the Lysis Plate 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 plate for 1 minute at maximum speed (up to 5,000 × *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 plate for 5 minutes at maximum speed (up to 5,000 × *g*) to pellet debris at the bottom of each well.

3. gDNA removal

- Carefully transfer up to 100 µL supernatant to a clean 96-well micro plate (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 plate before adding the supernatant. Cool at 4 °C before use.*
- *Seal plate with Adhesive Foil.*

- Incubate 10 minutes at room temperature.

4. *RNA purification*

- After gDNA removal is finished, transfer up to 80 µL of the lysate to the Purification Plate.

IMPORTANT NOTES:

- *Pipet slowly, drop-by-drop, and vertically onto the middle of the wells to not destroy the matrix surface (use an 8-channel pipette or robot).*
- *Do not touch the matrix bed with the pipette tip during sample loading!*

- Centrifuge the plate stack (Purification Plate on top of the Elution Plate) for 1 minute at 1,000 x *g*.
- Purified total RNA is in the flow-through and ready-to-use.

NOTE:

- *The supplied Adhesive Foil cannot be used for the storage of nucleic acids.*

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: 96-well plate kits

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

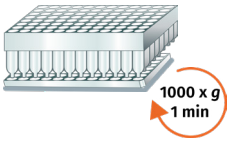
Preparation before starting

- 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. Purification Plate preparation

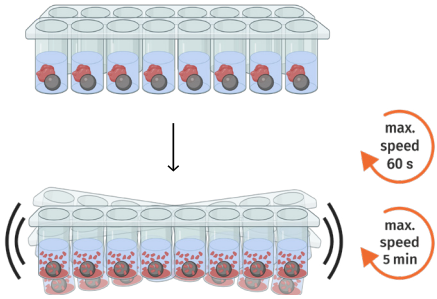
Purification Plate →

Conditioning Plate →



- Detach first the lower and then the upper foil from the Purification Plate.
- Place the Purification Plate on top of the Conditioning Plate.
- Centrifuge plate stack for 1 min at 1,000 x *g*.
- Discard the flow-through.
- Place the Purification Plate on top of the Elution Plate.

2. Homogenization and lysis

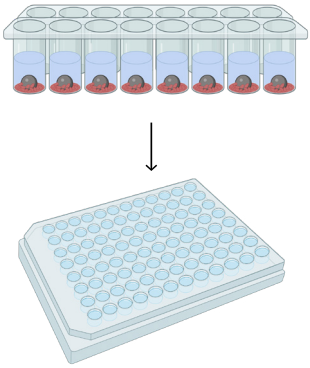


- Place one steel bead and a tissue sample in each well of the Lysis Plate.
- Add 150 µL LB.
- Seal wells with Cap Strips.
- Bead beat 4 x 30 sec at 30 Hz.
- Centrifuge 60 sec max. speed.
- Incubate at 40 °C for 5 min at 1,400 rpm.
- Add 22 µL Clearing Solution and mix.
- Centrifuge 5 min at max. speed (up to 5,000 x *g*).

IMPORTANT NOTE:

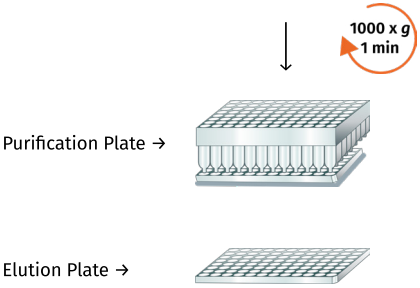
Longer incubation can lead to RNA degradation. Therefore, it is highly important to not prolong the incubation period.

3. gDNA removal



- Transfer 100 µL of cleared lysate to a micro plate.
- 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. 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 plant species 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 reduced release of RNA. Make sure to always resuspend 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 Purification Plate is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the well 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.
A_{260}/A_{280} value is low	**Wrong blank in measurements** Use supplied Low-TE Buffer (T) as blank and not RNase free water in measurements. **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.
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/–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.

Degraded RNA (continuation)

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 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.

Tilted matrix in column	**Incorrect storage of Purification Plate** If you observe that the matrix is tilted and not flat after centrifugation, the Purification Plate was stored incorrectly. Always make sure that the Purification Plates are stored in the right position (label facing up).
Poor performance in downstream experiments	**Tilted matrix bed** A tilted matrix bed can lead to inappropriate sample flow through and therefore insufficient time of interaction with the matrix surface, which can lead to poor extraction performance. If you observe that the matrix is tilted and not flat, the Purification Plate was stored incorrectly. Always make sure that the Purification Plates are stored in vertical position (label facing up). **Loading of Purification Plate** The correct loading of the Purification Plate is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the well 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 stick to the correct time mentioned in the protocol to avoid insufficient passage through the matrix bed.
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.

Occurrence of cross-contamination (continuation)

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.

Centrifuge settings

Excessive deceleration of the centrifuge may lead to cross-contamination of the samples from one well into the other. Experience shows that this problem only occurs with deceleration times of 2–3 seconds. Ideally, the deceleration of the centrifuge should take between 15–20 seconds.

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.

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!

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