EchoLUTION™ Cell Culture RNA Kit

Plate kits

USER MANUAL

REF

011-114-002

011-114-008

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

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1. INTENDED PURPOSE

The BioEcho EchoLUTION Cell Culture RNA Kit is intended for easy, rapid, and efficient RNA extraction from human and animal cultured cells. The excellent yield and purity of total RNA obtained with the EchoLUTION Cell Culture RNA Kit allows use in downstream applications without further processing.

The EchoLUTION Cell Culture RNA Kit is intended for research use only.

2. EXPLANATION OF THE KIT

The EchoLUTION Cell Culture RNA Kit is characterized by the EchoLUTION single-step purification technology and an ultra-fast lysis step. Together, they reduce the lysis step to 5 minutes and the overall extraction time with consistent sensitivity compared to state-of-the-art methods.

The EchoLUTION Cell Culture RNA Kit benefits are:

- Short processing time
- Ultra-fast lysis
- Few protocol steps
- High sample throughput with minor equipment and capital investment
- 60 % less plastic waste compared to silica-based products
- Less toxic reagents

For further details about kit specifications see Table 1.

Table 1: Kit specifications

Specification	Description
Sample input	Cell culture
Sample condition	Fresh or stored
Max. number of cells	2×10^6 cells
Purified nucleic acid	Total RNA
Elution volume	100 µL
Expected yields	Up to 30 µg (depends on amount of starting material)

2.1. Extraction principle

The key steps of the EchoLUTION Cell Culture RNA extraction protocol are:

1. Lysis and transfer

The ultra-fast lysis is a non-enzymatic reaction that guarantees the immediate lysis of cells. Additionally, it inactivates nucleases and stabilizes the nucleic acids during a 5-minutes incubation period.

2. Single-step purification

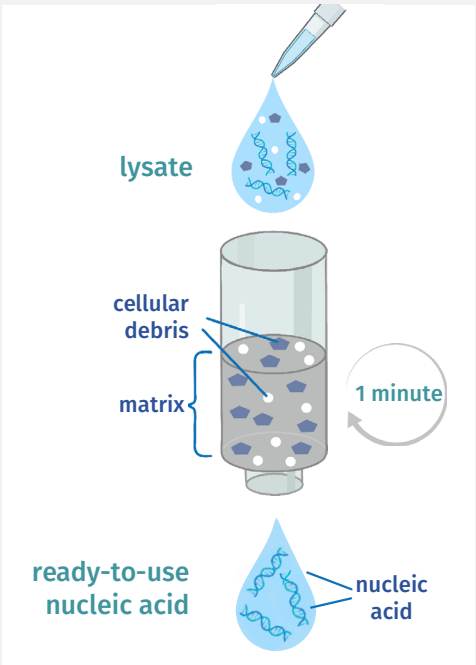
Once the cleared lysate is transferred onto the purification plate, it is purified in a one-minute centrifugation step. The RNA passes through the purification matrix without further interaction while impurities and cellular debris are held back and removed.

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.



3. Ready-to-use RNA

The isolated RNA is ready-to-use for standard downstream applications such as qPCR and sequencing.

2.2 General comments

Comparison of the EchoLUTION technology to silca technologies – General aspects and handling

Using the EchoLUTION technology, nucleic acids are not bound to a membrane or magnetic beads and can migrate freely through the filter matrix. Unwanted components of the lysate are removed from the sample and remain 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 washed away by repeated washing with chaotropic and alcohol-containing wash buffers. Finally, 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 RNA

For high quality RNA extractions, the samples must be Ribonuclease (RNase)-free, as they digest RNA very efficiently. In general, these enzymes are very active, stable, and difficult to inactivate. Even small amounts can digest the RNA and lead to insufficient experimental outcome. To avoid RNase contamination of your samples some things need to be considered. Always eliminate possible RNase contaminations from any consumables before starting your extractions. To not introduce RNases into the samples the working area needs to be RNase-free throughout the whole procedure.

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

In general, working with cautious microbiological, sterile practices 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-time storage purified RNA may be stored at –70 °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.

Handling of Purification Plate

The EchoLUTION purification matrix is a chromatographic column. Chromatographic 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).

To guarantee proper handling of the chromatography columns be aware 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 below or the QR code:

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

For support on suitable centrifuges, please contact BioEcho.

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 are:

- RNA and DNA content of the cell type
- Cell numbers: Higher cell numbers than 2×10^6 cells per sample could lead to handling difficulties and inhibition in downstream experiments.
- Volume of lysis buffer and insufficient lysis leads to low RNA recovery.
- Extended lysis time leads to degradation of the RNA

Lysing and homogenizing starting material

The efficient lysis and homogenization of the starting material is an asset for successful extraction of total RNA from cell cultures.

Correct lysis of the cells is important to release the RNA from the cells and release all the RNA contained in the sample. Incomplete lysis results in drastically reduced RNA yields.

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 and contamination with genomic DNA.

The homogenization of the sample is so important to reduce the viscosity of the lysates, to ensure precise handling and pipetting. Remaining cell clumps can lead to reduced RNA yield and handling issues.

Tilted matrix after conditioning

If you observed that the matrix is tilted and not flat after centrifugation, we recommend prolonging the standing time to up to 30 min before conditioning.

3. MATERIALS

3.1. Materials provided

The kit contains a lysis buffer and a lysis solution that require preparation before using them for the first time. Please read section **4.1** before starting your RNA extraction.

Table 2: *Kit content EchoLUTION Cell Culture RNA Kit, plate format*

Product number	011-114-002	011-114-008
Reactions	2 x 96	8 x 96
Lysis Buffer Cell RNA concentrate* (LB)	275 mg	4 x 275 mg
Lysis Solution Cell RNA* (LS)	20 mL	4 x 20 mL
Clearing Solution Cell RNA (CS)	2.9 mL	12 mL
Low-TE Buffer (T)	Blank only	Blank only
Purification Plate 96 Type 4	2	8
Elution Plate 96 Type 1	2	8
Adhesive Foil	2	8

**For correct preparation of these components, read section 4.1 Kit and reagents in the storage and stability chapter.*

3.2. Materials required but not provided

A. Conical centrifugation tubes

For the preparation of Master Mixes tubes.

B. Multichannel reagent reservoir

For pipetting of prepared Master Mixes reagent reservoirs for multichannel pipettes are needed.

C. Conditioning plate

The conditioning plate is necessary to remove the matrix storage buffer of the purification plate. The conditioning plate can be re-used up to 20-times and 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.

D. Lysis plate

The lysis plate necessary for mixing the Lysis Buffer with the sample (see section 2.1 Purification principle step 1) is not included in the kit. BioEcho offers a suitable lysis plate for sale (product number 060-004-008). However, you can also use your lysis plate of choice. In this case, the lysis plate should be a 96-well plate with a capacity of at least 500 µL per well.

E. Plates for counterbalance in centrifuge

In case an odd number of plates is processed, please use a plate sandwich of similar height and fill wells with an appropriate amount of water as tare.

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.
- At least 1,000 x *g*
- Minimum needed plate holder height is 5 cm.
- Swing out rotor.

B. Pipetting equipment

Pipetting can be performed using a single-channel pipette as well as a multi-channel pipette.

C. Thermal shaker for 96-well plates

A 96-well plate thermal shaker with agitation (up to 1,400 rpm), capable of heating up to 40 °C is used for the lysis step. Alternatively: Heating Block or heat chamber.

D. Vortex mixer

A vortex mixer is required for lysate mixture.

4. STORAGE AND STABILITY

4.1. Kit and reagents

- The EchoLUTION Cell Culture RNA Kit is shipped at ambient temperature.
- The purification plates and the Lysis Buffer Cell RNA (LB) are stable at 2 – 8 °C until the expiration date printed on the label of the component. The other kit components are stable at room temperature (15 – 25 °C) for at least a year.
- Store the purification plates in vertical position (label facing up).
- Longer storage in the refrigerator or at room temperature is not recommended!

Before starting any RNA extraction with the **EchoLUTION Cell Culture RNA Kit (REF: 011-114-002 and REF: 011-114-008)** prepare the following.

- Add the complete Lysis Solution Cell RNA (LS) to the Lysis Buffer Cell RNA (LB) (brown bottle). Mix by inversion around 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 Cell RNA will be stable for six months when stored at -20 °C. If storage is not possible at this temperature, the Lysis Buffer Cell RNA can be stored at 2 – 8 °C for two weeks (mark date on the bottle to make sure buffer is not expired).

4.2. Sample collection

A. Handling frozen cells for RNA extraction

Keep samples at -70 °C until you are ready to begin

If samples cannot be processed immediately, dry cell pellets or cells resuspended in lysis buffer can be stored at -70°C until usage. It is important to avoid freeze/thaw cycles without protection by lysis reagents or the RNA will be degraded.

For freezing the cell numbers need to be determined first, to make sure that cell input is adjusted to protocol requirements. When thawing the samples, the pellets should be slightly thawed and dislodged by flicking the tube to loosen the cell pellet before adding the lysis buffer in **step 2**.

4.3. Storage and stability of purified nucleic acids

For long-term storage of purified RNA, we recommend to store the RNA samples at -70 °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 BioEcho for the SDS.

Component	Hazard component	GHS symbol	Hazard statements	Precautionary statements	Aditional statement
Clearing Solution (CS)	Strontium chloride	 Danger	H318	P101; P102; P103; P280 P305+P351+P338 P310; P501	-
Lysis Buffer (LB*)	Tris	 Danger	H314; H318	P260; P280; P303+P361+P353; P304+340+310; P305+P351+P338; P363	-

*Note: After addition of Lysis Solution, concentration of TCEP is below critical and no precautionary measures need to be taken.

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.

P304+340+310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.

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.

P363: Wash contaminated clothing before reuse.

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 the waste disposal company, where you will 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 BioEcho for the SDS.

Dispose of biological samples and all liquid waste generated during the purification procedure as biohazardous waste.

A. Components and purification plates

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

7. PROTOCOL

This protocol has been developed to purify total RNA from cultured cells using the EchoLUTION Cell Culture RNA Kit.

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

Preparation before starting:

- Prepare the Lysis Buffer Cell RNA (LB) by adding the complete Lysis Solution Cell RNA (LS) to the Lysis Buffer Cell RNA (LB) (brown bottle). Mix by inversion around 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 Cell RNA will be stable for six months when stored at -20 °C. If storage is not possible at this temperature, the Lysis Buffer Cell RNA can be stored at 2 – 8 °C for two weeks (make sure to note date on LB bottle).
- Prepared buffer LB may form precipitates upon storage below RT. Before use redissolve by allowing it to warm up to room temperature for minimum 10 minutes.
- Pre-heat the thermal plate shaker to 40 °C
- Set centrifuge to 1,000 x *g*
- Carry out the complete RNA extraction at room temperature.

IMPORTANT NOTE:

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

1. *Purification plate preparation*

- Detach first lower and then upper foil from the purification plate. Please keep the plates in a horizontal position while removing the foils, because the wells contain liquid.
- Place purification plate on top of conditioning plate (not provided, product number 060-001-002 or 060-001-008).

- Centrifuge 1 min at 1,000 x *g*, discard flow-through.
- Place purification plate on top of elution plate.
- Proceed directly with step 2.

NOTE:

- *The centrifuge rotor needs to hold plate sandwiches of 5 cm. Conditioning plates can be reused.*

2. Sample preparation and lysis

a) Suspension cells

- Harvest up to 2×10^6 cells in a 0.5 mL microplate (not provided, product number 060-004-008) or into 1.5 mL reaction tube. Pellet cells by centrifugation at 500 x *g* for 5 min and remove the complete supernatant carefully.
- Add 100 µL LB to the prepared cells
- Incubate lysate at 40 °C in a thermal shaker with constant shaking of 1,400 rpm for 5 min (longer lysis time can lead to degradation of the RNA).

NOTE:

- *Ensure removal of the complete medium, as lysis can be inhibited by left over culture medium and thereby reduce RNA yield and quality.*
- *Avoid disturbing the cell pellet while removing the media, as cell loss leads to reduced RNA yields.*
- *If lysates are in a 0.5 mL micro plate, attach adhesive foil to the plate before incubating. When working with microplates below 0.5 mL volume the use of non-absorbent sealing foils is recommended (not provided, e.g. 050-008-050) before incubating.*

b) Adherent cells

- Use up to 2×10^6 cells per reaction.
- If cells are grown in a 96 well cell culture plate cell can be lysed directly in the well. If cells are grown in other cell culture vessels, cells must be trypsinized and pelleted by centrifugation before lysis.

Direct lysis:

- Completely aspirate the cell culture medium.
- Add 100 µL LB to the prepared cells.
- Incubate lysate at 40 °C in a thermal shaker with constant shaking of 1,400 rpm for 5 min (longer lysis time can lead to degradation of the RNA).

NOTE:

- *Do not use adhesive foil for closing the cell culture plate, as well volume is too low and could cause loss of lysate, due to the absorbent material of the foil.*
- *Make sure that lysis buffer covers the complete cell layer to ensure complete lysis.*

Trypsinization

- Remove the culture medium and wash with PBS.
- Aspirate the PBS and add 0.1 – 0.25 % trypsin in PBS and follow your trypsinization protocol.
- Collect all cells from dish or flask and inactivate trypsin by adding medium (containing serum).
- Transfer cells into a 0.5 mL microplate (not provided, product number 060-004-008) or into 1.5 mL reaction tubes (not provided) and centrifuge at 500 x *g* for 5 min.
- Completely aspirate the supernatant.
- Add 100 µL LB to the prepared cells.
- Incubate lysate at 40 °C in a thermal shaker with constant shaking of 1,400 rpm for 5 min (longer lysis time can lead to degradation of the RNA).

NOTE:

- *Ensure removal of the complete medium, as lysis can be inhibited by left over culture medium and thereby reduce RNA yield and quality.*
- *Make sure to wash off trypsin, as leftover trypsin can reduce RNA yield and quality.*
- *Transfer all cells to the reaction tube or lysis plate, as cell loss leads to reduced RNA yields.*
- *If lysates are in a 0.5 mL micro plate, attach adhesive foil to the plate before incubating. When working with microplates below 0.5 mL volume the use of non-absorbent sealing foils is recommended (not provided, e.g. 050-008-050) before incubating.*

3. *RNA Purification*

- Remove foil of the lysis plate and add 15 µL Clearing Solution (CS) to each sample.
- Mix sample by pipetting and transfer up to 100 µL of the lysate to the purification plate.

IMPORTANT NOTE:

- *For mixing and transfer of lysate to the purification plate the usage of wide bore tips is recommended.*
- *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 loaded purification plate on top of elution plate for 1 min at 1,000 x *g*.
- Purified RNA is in the flow-through and ready-to-use.

NOTE:

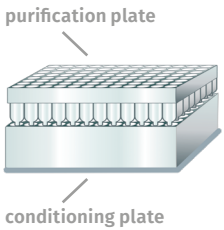
- *The supplied adhesive foil cannot be used for the storage of nucleic acids.*

The extracted RNA can be stored or used directly. For long-term storage, place your RNA samples at -70 °C. For spectrophotometric analysis, use the Low-TE Buffer supplied with the kit as blank.

7.1. Quick protocol

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

1. Purification plate preparation

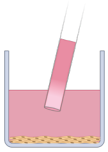


1000 x *g*
1 min

- Detach first lower and then upper foil from purification plate.
- Place purification plate on top of conditioning plate.
- Centrifuge and discard flow-through.
- Place purification plate on top of elution plate.

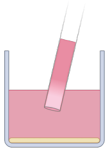
2. Sample preparation and lysis

Suspension cells



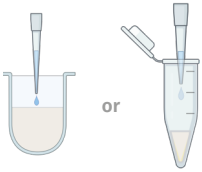
- Transfer cells to lysis plate or tube.
- Spin and remove culture media.
- Add 100 µL LB and resuspend cells.
- Incubate plate or tube at 40 °C for 5 min at 1,400 rpm.

Adherent cells

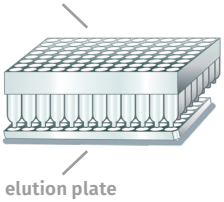


- Aspirate media.
- Lyse directly on plate or trypsinze (transfer to lysis plate).
- Add 100 µL LB to the cells.
- Incubate plate or tube at 40 °C for 5 min at 1,400 rpm.

3. RNA purification



- Add 15 µL CS.
- Mix by pipetting up and down.



1000 x *g*
1 min

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

8. QUALITY CONTROL

Following BioEcho's Quality Management System, each lot of EchoLUTION Cell Culture RNA Kit is tested against predetermined specifications to ensure consistent product quality.

9. TROUBLESHOOTING

Obversation	Comments and suggestions
RNA yield and concentration is low	**Low RNA content of the used cell type** Some cells have very low RNA yield. Also, the culturing conditions can lead to variations in RNA content. **Sample input** Always use correct number of cells as starting material (up to 2×10^6 cells per sample) to ensure appropriate experimental conditions. **Incomplete removal of cell-culture medium** Leftover cell culture medium can lead to inhibition of the lysis reaction and thereby low RNA yields. Always ensure complete removal of the cell culture medium, see protocols. **Insufficient lysis of starting material** Incorrect lysis of the starting material results in drastically reduced RNA yields. If lysis is incomplete the cells do not release all contained RNA of the sample. Make sure to stick to the correct lysis buffer volumes and remove medium components carefully. **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 well/cell layer when working with adherent cells, to ensure lysis of all cells.

	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, 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 RNA.
A260/A280 value is low	**Wrong blank in measurements** Use supplied Low-TE Buffer (T) as blank and not RNAse free water in measurements. **Sample input** Always use up to 2×10^6 cells per sample to ensure appropriate experimental conditions, in future extractions reduce the cell number
Degraded RNA	**Incorrect storage of cells** Extension of storage time can lead to RNA degradation. Make sure to store cells in RNA stabilizationreagent according to manufacturer's recommendations. Frozen cell pellets should be stored at -70°C until usage. Important is to avoid freeze/thaw cycle without protection by stabilizers, see also chapter 4.2. **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 chapter 2.2 for detailed instructions. It is highly recommended to use specific workplaces and equipment that have not been used in DNA preparations including RNAse digests. Always keep samples cold to ensure high quality RNA extraction.

	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.
DNA contamination	**Too much sample input** Do not use more than 2×10^6 cells per sample to ensure appropriate experimental conditions, eventually further reduce the cell number. **Low RNA content of cells** Some cells have very low RNA content, which can lead to inappropriate extraction conditions. Also, the culturing conditions can lead to variations in RNA content. **Incorrect lysis conditions** Longer lysis time and temperature can lead to contamination with genomic DNA. Stick to temperature and time mentioned in the protocol. **Optimal procedure not used** In general, the spin column eliminates most DNA. However, certain sensitive RNA downstream applications may require further DNA removal. A gDNA Removal Mix will soon be available from BioEcho.
Tilted matrix in column	**Incorrect storage of purification plate** If you observed 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 vertical position (label facing up).
Poor performance in downstream experiments	**Tilted resin bed** A tilted resin 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 observed 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** Do not use more than 2×10^6 cells per sample to ensure appropriate experimental conditions, eventually further reduce the cell number.
Occurrence of cross-contamination	**Contaminated pipettes** The use of contaminated pipettes can lead to cross-contaminations. BioEcho recommends 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, working with cautious microbiological, sterile practices should always be used 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.
Eluate is missing or volume to low	**Sealing of purification plate** The use of non-air-permeable foils create a vacuum inside the plate during centrifugation, which leads to inadequate elution. Do not seal the purification plate when eluting the RNA. If sealing is needed air-permeable adhesive foil can additionally be ordered from BioEcho (product number 050-007-050). **Centrifuge settings** Low centrifugation setting can lead to inadequate elution. Most centrifuges offer the choice between rpm and *g*-force (rcf); if not, 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 RNA.

For questions and further troubleshooting, please contact us!

10. LIMITATIONS OF USE

Limitations regarding EchoLUTION Cell Culture RNA Kit are listed as follows:

- Strict compliance with the user manual is required for RNA purification. Following good laboratory practices is crucial for the successful usage of the product. Appropriate handling of the reagents is essential to avoid contaminations or impurities.
- The RNA yield varies and is dependent on several factors including the technique of the person taking the sample.
- The proof of principle for the EchoLUTION Cell Culture RNA Kit was evaluated and confirmed using state-of-the-art RT-PCR 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 Cell Culture RNA products and on this user manual.

Symbols	Description
	Manufacturer
	Product number
	Batch code
	Temperature limitation
	Do not re-use
	Expiration date
	Consult instructions

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!

+49 221 9988 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