

# EchoLUTION™ Cell Culture RNA Kit

## Spin column kits

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# USER MANUAL

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**REF**

**011-114-050**

**011-114-250**



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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 cell cultures. 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. 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 <sup>6</sup> 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-minute incubation period.

### 2. Single-step purification

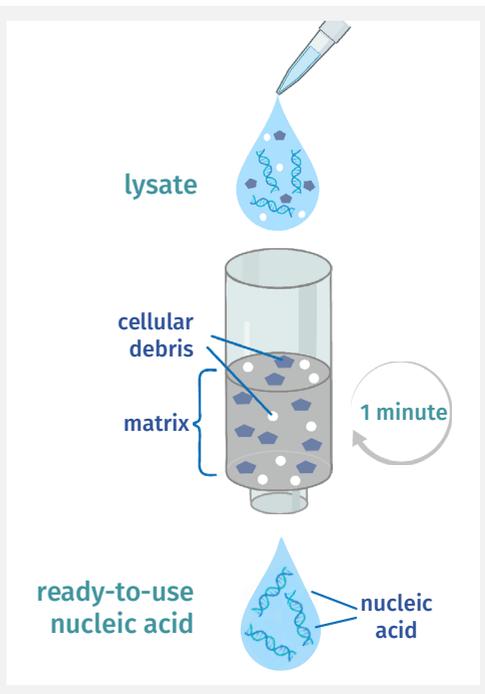
Once the cleared lysate is transferred onto the spin column, it is purified in a one-minute centrifugation step. The RNA passes through the purification matrix without further interaction while impurities and cellular debris are held back and removed.

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 downstream applications such as qPCR and sequencing.

## 2.2. General comments

### ***Comparison of the EchoLUTION technology to silica technologies – General aspects and handling***

Using the EchoLUTION technology, nucleic acids are not bound to a membrane or magnetic beads and can migrate freely through the filter matrix. Unwanted components of the lysate are removed from the sample 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. Eventually, the nucleic acids are eluted with an aqueous buffer in the desired volume. Due to the repeated washing steps, silica-based methods are time-consuming, labor-intensive, and environmentally unfriendly.

### ***Handling 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  $-70\text{ }^{\circ}\text{C}$ . 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 columns***

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](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  $2 \times 10^6$  cells per sample could lead to handling difficulties and inhibition in downstream experiments.
- Inaccurate 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. However, if you want to avoid this step, we suggest using a swing-out rotor centrifuge with the Spin Column Adapter for Plate Centrifuges offered by BioEcho ([product number: 050-011-024](#)).

## 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:** Content of EchoLUTION Cell Culture RNA Kit

Product number	011-014-050	011-014-250
Reactions	50	250
Lysis Buffer Cell RNA* (LB)	1 x 75 mg	2 x 180 mg
Lysis Solution Cell RNA* (LS)	1 x 5 mL	2 x 13 mL
Clearing Solution Cell RNA (CS)	1 x 0.8 mL	1 x 3.8 mL
Low-TE Buffer (T)	1 tube	1 tube
Spin Columns Cell RNA	50	250

\* 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. Microcentrifuge tubes

Use a 2 mL tube for spin column preparation, and a 1.5 mL tube for sample lysis and elution.

### 3.3 Laboratory equipment needed

#### A. Microcentrifuge

Centrifugation can be performed in a microcentrifuge with a rotor for 2 mL reaction tubes. The centrifuge needs to reach a speed of 1,000 x g. When using a plate centrifuge, please use the Spin Column Adapter for Plate Centrifuges offered by BioEcho ([product number: 050-011-024](#)).

#### B. Pipetting equipment

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

#### C. Standard laboratory heat shaker

A heat shaker is used for the lysis step. It needs to reach up to 40 °C and 1,400 rpm (e.g., Eppendorf® ThermoMixer® C).

#### D. Vortex mixer

A vortex mixer is required for lysate mixture.

## 4. STORAGE AND STABILITY

### 4.1. Kit reagents

- The EchoLUTION Cell Culture RNA Kit is shipped at ambient temperature.
- Upon kit arrival, Lysis Buffer Cell RNA (LB) should be stored at 2 – 8 °C. The other kit components are stable at room temperature (15 – 25 °C) for at least one year.

Before starting any RNA extraction with the **EchoLUTION Cell Culture RNA Kit (REF: 011-014-050 and REF: 011-014-250 )** 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 for until usage. It is important is 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 recommended 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	Additional statement
Clearing Solution (CS)	Strontium chloride	 Danger	H318	P101; P102; P103; P280 P305+P351+P338 P310; P501	-
Lysis Buffer (LB*)	Tris (2-carboxyethyl) phosphinhydrochlorid	 Danger	H314; H318	P260; P280; P303+P361+P353; P304+340+310; P305+P351+P338; P363	-

### Hazard Statements

**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.

**P280:** Wear eye protection/face protection.

**P305+P351+P338:** *IF IN EYES:* Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

**P310:** Immediately call a *POISON CENTER*/doctor.

**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 spin columns***

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

## 7. PROTOCOL

This protocol has been developed to purify total RNA from human and animal cell cultures using the EchoLUTION Cell Culture RNA Kit.

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



### **Preparation before starting:**

- Vortex EchoLUTION Spin Columns thoroughly, to homogenize the resin and remove air bubbles. Then place them in a 2 mL reaction tube (not provided) and let them stand for at least 10 min.
- 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 15 °C. Before use redissolve by allowing it to warm up to room temperature for minimum 10 minutes.
- Prepare 1.5 mL microcentrifuge tubes (not provided, preferably safe lock) for each sample.
- Pre-heat the thermal shaker to 40 °C.
- Set microcentrifuge 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. Spin column preparation**

- Loosen the cap of the spin column ½ a turn and snap off the bottom.
- Place the spin column in a 2 mL reaction tube (not provided).
- Centrifuge 1 min at 1,000 x g, and discard flow-through.
- Place the spin column in a fresh 1.5 mL reaction tube (not provided).



**ALTERNATIVELY:** You can use the BioEcho Cap Puncher to open the column (not supplied. Product ordering 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 min at 1,000 x g, discard flow-through and place the spin column in a fresh 1.5 mL reaction tube.

**NOTE:** Proceed directly with **step 2**.



## 2. Sample preparation and lysis

### a) Suspension cells

- Harvest up to  $2 \times 10^6$  cells in a 1.5 mL reaction tube (not provided). Pellet cells by centrifugation for 5 min at 500 x g and remove the complete supernatant carefully.
- Loosen the cell pellet thoroughly by flicking the tube.
- Add 100  $\mu$ L of prepared LB to each sample and resuspend cell pellet by vortexing. Make sure that no cell clumps are visible before proceeding.
- Incubate samples 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 hereby reduce RNA yield and quality.
- Avoid disturbing the cell pellet while removing the media, as cell loss leads to reduced RNA yields.
- Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields.
- Incomplete homogenization might lead to reduced RNA yields due to inefficient lysis.
- With cell numbers the viscosity of the lysate increases.
- During the resuspension, generation of foam is normal.

### b) Adherent cells

- Use up to  $2 \times 10^6$  cells per reaction.
- Depending on the culture vessel cells can be lysed directly in the well (96-well plate) or trypsinized and pelleted by centrifugation before lysis. For cells grown in cell culture flasks always trypsinize.

**Direct lysis:**

- Completely aspirate the cell culture medium.
- Add 100  $\mu$ L of prepared LB directly to the monolayer.
- Incubate samples 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).
- Remove cells from the culture surface and transfer to 1.5 mL reaction tube (not provided).

### **Trypsinization:**

- Remove the culture medium and wash with PBS.
- Aspirate the PBS and add 0.1 – 0.25% trypsin in PBS and follow your common trypsinization protocol.
- Collect all cells from dish or flask and inactivate trypsin by adding medium (containing serum).
- Transfer cells to a centrifuge tube (not provided) and centrifuge for 5 min at 500 x g.
- Completely aspirate the supernatant, add 100 µL of prepared LB and vortex or to resuspend cell clumps. Make sure that no cell clumps are visible before proceeding.
- Incubate samples 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 leftover culture medium and thereby reduce RNA yield and quality.*
- *Make sure that lysis buffer covers the complete cell layer to ensure optimal lysis.*
- *Transfer all cells to the reaction tube, as cell loss leads to reduced RNA yields.*
- *Incomplete homogenization might lead to reduced RNA yields due to inefficient lysis.*



### **3. RNA Purification**

- Add 15 µL of Clearing Solution (CS) to the lysate and vortex shortly.
- Centrifuge for 2 min at max. speed.
- Open the spin column and transfer the lysis supernatant slowly to the prepared spin column (max. 100 µL).

### **IMPORTANT NOTE:**

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



- Close the cap of the spin column and loosen the cap again by ½ a turn.
- Centrifuge the loaded column for 1 min at 1,000 x g.
- Purified RNA is in the flow-through.

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.

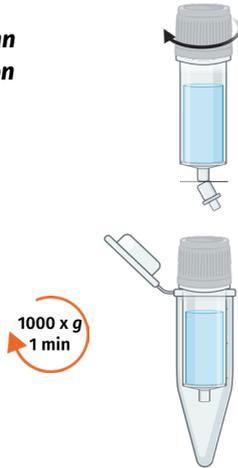
## 7.1. Quick protocol

### IMPORTANT NOTE:

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



### 1. Spin column preparation

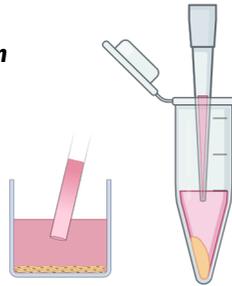


- Loosen the cap of the spin column ½ a turn snap off the bottom.

- 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 1,5mL reaction tube.



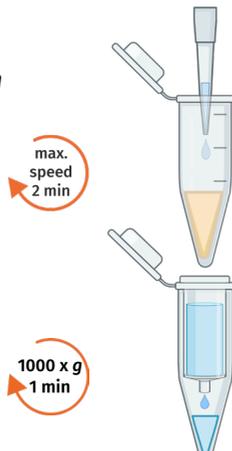
### 2. Sample preparation and lysis



- Harvest cells and remove culture media.
- Add 100 µL LB and resuspend cells.
- Incubate at 40 °C for 5 min at 1,400 rpm.



### 3. RNA purification



- Add 15 µL CS.
- Vortex shortly.
- Centrifuge for 2 min at max. speed.

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

## 8. QUALITY CONTROL

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

## 9. TROUBLESHOOTING

Observation	Comments and suggestions
<b>RNA yield and concentration is low</b>	<p><b>Low RNA content of the used cell type</b> 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.</p> <p><b>Sample input</b> Always use correct number of cells as starting material (up to <math>2 \times 10^6</math> cells per sample) to ensure appropriate experimental conditions.</p> <p><b>Incomplete removal of cell culture medium</b> 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.</p> <p><b>Insufficient lysis and homogenizing of starting material</b> Incorrect lysis and homogenization 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.</p> <p><b>Incorrect lysis conditions</b> Incorrect lysis and homogenization 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.</p> <p>Make sure to stick to the correct lysis buffer volumes and remove medium components carefully.</p>

### **Loading of column**

The correct loading of the spin column is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the spin column to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading.

### **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.

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### **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**

Do not use more than  $2 \times 10^6$  cells per sample to ensure appropriate experimental conditions, eventually further reduce the cell number.

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### **Degraded RNA**

#### **Incorrect storage of cells**

Extension of storage time can lead to RNA degradation. Make sure to store cells in RNA stabilization reagent 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. Avoid RNase contamination of your samples. The working area and materials need to be RNase free throughout the whole procedure, see [chapter 2.2](#) for detailed instructions.

We highly recommend 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.

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### **DNA contamination**

#### **Too much sample input**

Do not use more than  $2 \times 10^6$  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 DNA Removal Mix will soon be available from BioEcho.

**Do not use any other reagents or products for DNA removal.**

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### **Tilted matrix in column**

#### **Inappropriate handling of spin column**

The spin column was closed during centrifugations, and vacuum was generated. 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.

See [chapter 2.2](#) for further instructions.

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### **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 after centrifugation, we recommend prolonging the standing time to up to 30 min before conditioning, see also [chapter 2.2](#).

### **Loading of column**

The correct loading of the spin column is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the spin column to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading.

### **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.

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### **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.

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### **Eluate is missing or volume to low**

#### **Inappropriate handling of spin column**

The spin column was closed during centrifugations, and vacuum was generated. See [chapter 2.2](#) for further instructions.

#### **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.

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**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.
- **Successful DNA removal can only be achieved using the DNA Removal Mix (available soon)**

## 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!



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