EchoLUTION™ Cell Culture RNA Kit

Spin column kits

USER MANUAL

REF

011-014-050

011-014-250

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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 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, these steps reduce the overall extraction time, and result in 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
Maximum 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. *Single-step purification principle*

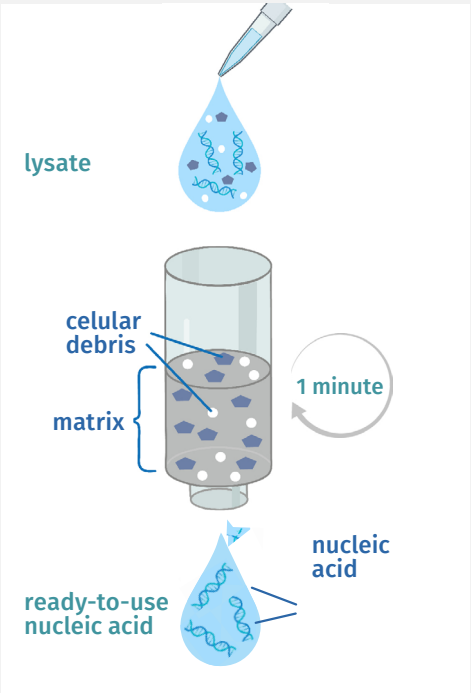
All BioEcho nucleic acid extraction kits are based on the EchoLUTION technology, which consists of tailored sample processing that includes lysis followed by single-step purification. The purification step works differently than conventional methods such as magnetic-bead and silica kits based on the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

The lysate is transferred to the Spin Column or Purification 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 technologies—general aspects and handling

Using 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 wash steps**
2. **Easy handling**
3. **Reduced plastic waste**

In contrast, silica technologies are based on the principle of concentration. Here, the nucleic acids present in the lysate bind to a silica surface (membrane, magnetic beads), while unwanted cell components are removed by repeated washing with chaotropic and alcohol-containing wash buffers. Eventually, the nucleic acids are eluted with an aqueous buffer in the desired volume. Due to the repeated washing steps, silica-based methods are time-consuming, labor-intensive and environmentally unfriendly.

Handling 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 nondisposable plasticware can be decontaminated using general laboratory reagents. Plasticware can be decontaminated with commercially available RNase removing solutions.

In general, working cautiously using sterile microbiological 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 matrix

The EchoLUTION purification matrix within the Spin Columns 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 Spin Column, 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.

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:

- Different cell types have different RNA and DNA content.
- Using more than 2×10^6 cells per sample could lead to handling difficulties and inhibition in downstream experiments.
- Incorrect volume of Lysis Buffer Cell RNA (LB) 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 important for successful extraction of total RNA from cell cultures.

Correct lysis of the cells is necessary to release the RNA from the cells, thus releasing all the RNA contained in the sample. Incomplete lysis results in drastically reduced RNA yields.

It is important to use the time and temperature mentioned in the protocol for the lysis step, as longer lysis time can lead to degradation of the RNA and contamination with genomic DNA.

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

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 2: ***Content of EchoLUTION Cell Culture RNA Kit, spin column format***

Product number	011-014-050	011-014-250
Product name	EchoLUTION Cell Culture RNA Kit (50)	EchoLUTION Cell Culture RNA Kit (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*

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 must be capable of reaching 1,000 x *g*. When using a plate centrifuge, please use our Spin Column Adapter for Plate Centrifuges (product number: 050-011-024).

B. Pipetting equipment

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

C. Standard laboratory heat shaker

Ideally, two thermal shaker with agitation (up to 1,400 rpm), capable of heating up to 40 °C are used for the lysis and the optional gDNA removal step (e.g.; Eppendorf® ThermoMixer® C). If just one thermal shaker is available, shut off heating after lysis step, as mentioned in the protocol. Alternatively, you can use a heating block or heat chamber.

D. Vortex mixer

A vortex mixer is required for lysate mixture.

E. Cap Puncher

Alternatively, the cap puncher can be used for convenient handling of Spin Columns. To purchase this item, use the product number 050-001-001.

F. Spin Column Adapter for plate centrifuges

If you want to use a plate centrifuge for spin columns and avoid the standing time of the Spin Columns mentioned in the protocol, we suggest using a swing-out rotor centrifuge with our Spin Column Adapter for Plate Centrifuges (product number: 050-011-024).

4. STORAGE AND STABILITY

4.1. Kit and 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 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 one year.

Before starting RNA extraction with the **EchoLUTION Cell Culture RNA Kit (REF: 011-014-050 and 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 inverting 5–10 times, then let stand for 10 minutes to reduce the foam. After mixing, indicate the addition of the Lysis Solution Cell RNA (LS) and the date. Prepared Lysis Buffer Cell RNA (LB) will be stable for six months when stored at –20 °C. If storage is not possible at this temperature, the Lysis Buffer Cell 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 Cell RNA (LS) or prepared Lysis Buffer Cell 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.

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 Cell RNA (LB) can be stored at –70°C until use. It is important to avoid freeze-thaw cycles as samples that do not contain stabilizing reagents can be degraded.

Before freezing, the cell numbers should be determined 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 Cell RNA (LB) in step 2.

4.3. Storage and stability of purified nucleic acids

For long-term storage of purified RNA, we recommend storing 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 us for the SDS.

Table 3: ***EchoLUTION Cell Culture RNA Kit safety information***

Component	Hazardous component	GHS symbol	Hazard statements	Precautionary statements	Additional statement
Clearing Solution Cell RNA (CS)	Strontium chloride	 Danger	H318	P101; P102; P103; P280; P305+P351+P338 P310; P501	-
Lysis Buffer Cell RNA (LB*)	Tris (2-carboxyethyl) phosphine hydrochloride	 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 breathe 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. Contact your waste disposal company to obtain information on laboratory waste disposal (waste code number 16 05 06). For further information, please refer to the instructions supplied with our SDS. Please contact us for the SDS.

Dispose of biological samples as biohazardous waste and all remaining liquid waste generated during the purification procedure according to local regulations.

A. Components and Spin Columns

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, and should be disposed of according to local /regional/national/international regulations.

7. PROTOCOL

This protocol has been developed to extract 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:

- Vortex EchoLUTION Spin Column thoroughly to homogenize the purification matrix and remove air bubbles. If necessary, flick or gently spin down by hand until it is free of air bubbles. Place each tube in a 2 mL reaction tube (not provided) and let them stand for at least 10 minutes.
- 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 inverting around 5–10 times, then let stand for 10 minutes to reduce the foam. After mixing, indicate the addition of the Lysis Solution Cell RNA (LS) and the date. Prepared Lysis Buffer Cell RNA (LB) will be stable for six months when stored at –20 °C. If storage is not possible at this temperature, the Lysis Buffer Cell RNA (LB) can be stored at 2–8 °C for two weeks (make sure to note date on LB bottle).
- Lysis Solution Cell RNA (LS) or prepared Lysis Buffer Cell RNA (LB) may form precipitates upon storage below room temperature. Before use, redissolve by allowing the buffer to warm up to room temperature for a minimum of 10 minutes or until precipitation is dissolved.
- Pre-heat the thermal shaker to 40 °C.
- Set the 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.*

1. Spin Column preparation

- Loosen the cap of the Spin Column by half a turn and snap off the bottom.
- Place the Spin Column in a 2 mL reaction tube (not provided).

1000 x *g*
1 min

- Centrifuge 1 minute at 1,000 x *g*, 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 minute 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 × 10^6 cells in a 1.5 mL reaction tube (not provided). Pellet cells by centrifugation for 5 minutes at 500 x *g*, and completely remove the supernatant carefully.
- Loosen the cell pellet thoroughly by flicking the tube.
- Add 100 µL of prepared Lysis Buffer Cell RNA (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 minutes (longer lysis time can lead to degradation of the RNA).

NOTE:

- *Ensure complete removal of the medium, as culture media can inhibit lysis, and thus 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.*
- *The viscosity of the lysate increases with the cell number.*
- *During the resuspension, generation of foam is normal.*

b) Adherent cells

- Use up to 2 × 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. Always trypsinize cells grown in cell-culture flasks.

Direct lysis:

- Completely aspirate the cell culture medium.
- Add 100 µL Lysis Buffer Cell RNA (LB) to the monolayer.
- Incubate samples at 40 °C in a thermal shaker with constant shaking of 1,400 rpm for 5 minutes (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 phosphate-buffered saline (PBS).
- Aspirate the PBS and add 0.1–0.25 % trypsin in PBS, and follow your common trypsinization protocol.
- Collect all cells from the dish or flask, and inactivate trypsin by adding medium (containing serum).
- Transfer cells to a centrifuge tube (not provided), and centrifuge for 5 minutes at 500 x *g*.
- Completely aspirate the supernatant, add 100 µL of prepared Lysis Buffer Cell RNA (LB), and vortex to resuspend cell clumps. Make sure that no cell clumps are visible before proceeding.
- Incubate lysate at 40 °C in a thermal shaker with constant shaking of 1,400 rpm for 5 minutes (longer lysis time can lead to degradation of the RNA).

NOTE:

- *Ensure complete removal of the medium, as culture media can inhibit lysis, and thus reduce RNA yield and quality.*
- *Make sure that Lysis Buffer Cell RNA (LB) completely covers the 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 Cell RNA (CS) to the lysate and vortex briefly.

- Centrifuge for 2 minutes at maximum speed.
- Open the Spin Column and slowly transfer the lysis supernatant to the prepared Spin Column (maximum 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 a half turn.

- Centrifuge the loaded column for 1 minute 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.

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

7.1. Quick protocol EchoLUTION Cell Culture RNA Kit: Spin Column kits

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

Preparation before starting

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

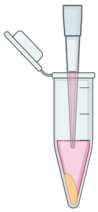
1. Spin Column preparation

- Loosen the cap of the Spin Column half a turn snap off the bottom.

1000 x *g*
1 min

- Place Spin Column in a 2 mL reaction tube.
- Centrifugate 1 min at 1,000 x *g*.
- Discard flow-through.
- Place Spin Column in a new 1.5 mL 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

max.
speed
2 min

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

1000 x *g*
1 min

- 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. ALTERNATIVE PROTOCOL

A. Materials provided

Table 4: ***Content of the gDNA Removal Mix, spin format***

Product number	011-901-050	011-901-250
Product name	gDNA Removal Mix (50)	gDNA Removal Mix (250)
Reactions	50	250
Solution A	300 µL	3 x 300 µL
Solution B	300 µL	3 x 300 µL

B. Materials required but not provided

- EchoLUTION Cell Culture RNA Kit (50 or 250).

C. Storage conditions

- Kit shipped at 4 °C. Store at –20 °C upon arrival.

8.1. Protocol with gDNA Removal Mix

This protocol has been developed as an additional option for extracting gDNA-free RNA from cultured cells using the EchoLUTION Cell Culture RNA Kit in combination with the gDNA Removal Mix (product number 011-901-050 for 50 reactions and 011-901-250 for 250 reactions).

The EchoLUTION Cell Culture RNA Kit removes most of the gDNA in the sample. This optional gDNA removal protocol was developed for applications for which it is important to further reduce gDNA content.

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

Preparation before starting:

- Vortex EchoLUTION Spin Column thoroughly to homogenize the purification matrix and remove air bubbles. If necessary, flick or gently spin down by hand until it is free of air bubbles. Place each column in a 2 mL reaction tube (not provided) and let them stand to sediment the matrix for at least 10 minutes.
- 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 inverting 5–10 times, and then let stand for 10 minutes to reduce the foam. After mixing, indicate the addition of the Lysis Solution Cell RNA (LS) and the date. Prepared Lysis Buffer Cell RNA (LB) is stable for six months when stored at –20 °C. If storage is not possible at this temperature, the Lysis Buffer Cell RNA (LB) can be stored at 2–8 °C for two weeks.

- Lysis Solution Cell RNA (LS) or prepared Lysis Buffer Cell RNA (LB) may form precipitate when stored below room temperature. Before use, redissolve by allowing the buffer to warm to room temperature for a minimum of 10 minutes or until it dissolves.
- Prepare 1.5 mL microcentrifuge tubes (not provided, preferably safe lock) for each sample.
- Pre-heat the thermal shaker to 40 °C.
- Carry out the complete RNA extraction at room temperature.

  IMPORTANT NOTE:

  - *Choose x g (RCF), NOT RPM, unless stated otherwise.*
  - *Make sure the Lysis Buffer Cell RNA (LB) is prepared and warmed up to room temperature.*
  - *Prepare a gDNA removal master mix for the number of samples with tenfold excess (according to Table 5), mix gently and keep cold.*

Number of samples	1	12 (+10%)	Your calculations
Suspension A	2.5 µL	33 µL	
Suspension B	2.5 µL	33 µL	
Final volume	**5.0 µL**	**66 µL**	

Table 5: ***gDNA removal master mix***

1. Sample preparation and lysis

a) Suspension cells

- Harvest up to 2 × 10^6 cells in a 1.5 mL reaction tube (not provided). Pellet cells by centrifugation for 5 minutes at 500 x *g*, and completely remove the supernatant carefully.
- Loosen the cell pellet thoroughly by flicking the tube.
- Add 100 µL of prepared Lysis Buffer Cell RNA (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 minutes (longer lysis time can lead to degradation of the RNA).

  NOTE:

  - *Ensure complete removal of the medium, as culture media can inhibit lysis, and thus 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.*
  - *The viscosity of the lysate increases with the cell number.*
  - *During the resuspension, generation of foam is normal.*
  - *If only one thermal shaker is available shut off heating after the lysis incubation to cool down thermal shaker for gDNA removal.*

b) Adherent cells

- Use up to 2 × 10^6 cells per sample.
- Depending on the culture vessel, cells can be lysed directly in the well (96-well plate) or trypsinized and pelleted by centrifugation before lysis. Always trypsinize cells grown in cell culture flasks.

Direct lysis:

- Completely aspirate the cell culture medium.
- Add 100 µL Lysis Buffer Cell RNA (LB) to the monolayer.
- Incubate samples at 40 °C in a thermal shaker with constant shaking of 1,400 rpm for 5 minutes (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 phosphate-buffered saline (PBS).
- Aspirate the PBS and add 0.1–0.25 % trypsin in PBS, and follow your common trypsinization protocol.
- Collect all cells from the dish or flask, and inactivate trypsin by adding medium (containing serum).
- Transfer cells to a centrifuge tube (not provided), and centrifuge for 5 minutes at 500 x *g*.
- Completely aspirate the supernatant, add 100 µL of prepared Lysis Buffer Cell RNA (LB), and vortex 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 at 1,400 rpm for 5 minutes (longer lysis time can lead to degradation of the RNA).

  NOTE:
  - *Ensure complete removal of the medium, as culture media can inhibit lysis, and thus reduce RNA yield and quality.*
  - *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.*

- *During the resuspension, generation of foam is normal.*
- *The viscosity of the lysate increases with the cell number.*
- *If only one thermal shaker is available shut off heating after the lysis incubation to cool down thermal shaker for gDNA removal.*

2. gDNA removal

- After lysis is finished, open tube, add 15 µL Clearing Solution Cell RNA (CS) to each sample and mix by vortexing!

- Centrifuge for 2 minutes at maximum speed.
- Add 5 µL of gDNA removal master mix to the lysates.
- Incubate in a thermal shaker with constant shaking at 300 rpm for 10 minutes at room temperature.

NOTE:

- *When adding the gDNA removal master mix to the samples, make sure to not touch the pellet.*
- *If only one thermal shaker is available, you can turn off the heat after the lysis incubation step and incubate the samples in the warm shaker, up to 40 °C.*
- *During incubation, proceed with step 3.*

3. Spin Column preparation

- Loosen the cap of the Spin Column a half turn and snap off the bottom.
- Place the Spin Column in a 2 mL reaction tube (not provided).

- Centrifuge 1 minute at 1,000 x *g*, and discard flow-through.
- Place the Spin Column in a fresh 1.5 mL reaction tube (not provided).

ALTERNATIVE:

You can use the BioEcho Cap Puncher to open the column (not supplied, product number 050-001-001). To use the cap puncher correctly, punch a hole in the column cap, and lift the column together with the cap puncher out of the 2 mL reaction tube. Snap off the bottom closure of the column and detach the cap puncher. Place the punched Spin Column back into the 2 mL reaction tube. Centrifuge 1 minute at 1,000 x g, discard flow-through and place the Spin Column in a fresh 1.5 mL reaction tube.

4. *RNA Purification*

- Open the Spin Column and transfer the supernatant slowly to the prepared Spin Column (maximum 100 µL).

  IMPORTANT NOTES:

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

- Close the cap of the Spin Column and loosen the cap with a half turn.
- Centrifuge the loaded column for 1 minute at 1,000 x *g*.
- Purified RNA is in the flow-through.

The extracted RNA can be stored or used directly in downstream applications. For long-term storage, place your RNA samples at –70 °C.

IMPORTANT NOTE:

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

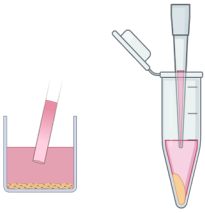
8.2. Quick protocol EchoLUTION Cell Culture RNA Kit with gDNA Removal Mix

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

Preparation before starting

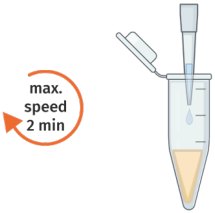
- Prepare the Lysis Buffer Cell RNA (LB).
- Pre-heat the thermal shaker to 40 °C.
- Set the microcentrifuge to 1,000 x *g*.

1. Sample preparation and lysis



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

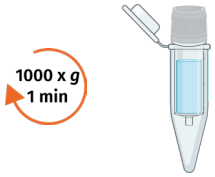
2. gDNA removal



- Prepare a gDNA removal master mix.
- Add 15 µL CS and vortex.
- Centrifuge for 2 minutes at maximum speed.
- Add 5 µL of DNA removal master mix.
- Incubate 10 minutes at room temperature and 300 rpm.

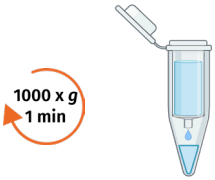
3. Spin Column preparation

- Loosen the cap of the Spin Column half a turn snap off the bottom.



- Place Spin Column in a 2 mL reaction tube.
- Centrifugate 1 minute at 1,000 x *g*.
- Discard flow-through.
- Place Spin Column in a new 1.5 mL reaction tube.

4. RNA purification



- Transfer up to 100 µL cleared lysate. Pipet slowly, drop-by-drop, onto the middle of the column without touching the matrix.
- Centrifuge 1 minute 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

Observation	Comments and suggestions
RNA yield and concentration is low	**They cell type used has low RNA content** 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. In case only a few cells are available for RNA extraction the reaction volume can be reduced by 50 % (reduction of all buffer volumes: Lysis Buffer Cell RNA (LB), Clearing Solution Cell RNA (CS), gDNA Removal Suspension A and Suspension B). **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 use the correct Lysis Buffer Cell RNA (LB) volumes and remove culture medium components carefully. **Incorrect lysis conditions** Incorrect lysis and homogenization of the starting material results in drastically reduced RNA yields.

RNA yield and concentration is low (continuation)	If lysis is incomplete, the cells do not release all the RNA contained in the sample. Make sure to use the correct Lysis Buffer Cell RNA (LB) volumes and remove medium components carefully. Lysis Solution Cell RNA (LS) and prepared Lysis Buffer Cell RNA (LB) may form precipitates upon storage below room temperature. Before use redissolve by allowing it to warm up to room temperature for minimum 10 minutes or until it is dissolved. **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 rcf is not available, calculate the rpm; see section 2.2. Always make sure to use the correct time mentioned in the protocol to avoid insufficient elution of RNA.
A_{260}/A_{280} value is low	**Wrong blank in measurements** Use supplied Low-TE Buffer (T) as blank for measurements and not RNase-free water. **Sample input** Always use up to 2×10^6 cells per sample to ensure appropriate experimental conditions. If this amount of cells is too much, repeat with fewer cells.
Degraded RNA	**Incorrect storage of samples** Extension of storage time can lead to RNA degradation. Make sure to store cells in RNA stabilization reagent according to manufacturer's recommendations.

Degraded RNA (continuation)

Frozen cell pellets should be stored at –70°C until use. Important is to avoid freeze-thaw cycle without protection by stabilizers, see also section 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 section 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 use the 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. If this amount of cells is too much (due to high nucleic acid content), reduce the number of cells used.

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 gDNA. Use the temperature and time mentioned in the protocol.

DNA contamination (continuation)	**Optimal procedure not used** In general, the Spin Column eliminates most *g*DNA. However, certain sensitive RNA downstream applications may require further gDNA removal. Use the gDNA Removal Mix, product number 011-901-050 for 50 reactions and 011-901-250 for 250 reactions. **Do not use any other reagents or products for gDNA removal.**
Tilted matrix in column	**Inappropriate handling of Spin Column** If the Spin Column was closed during centrifugation, a vacuum could be generated. Also highly tilted matrix after conditioning can lead to insufficient time of interaction with the matrix, which can result in a poor extraction performance. However, when using centrifuges with a fixed angle rotor, the matrix does not become completely horizontal after conditioning. A slightly tilted surface according to the angle of the rotor (usually 30°) is to be expected. This does not limit the purification ability. But if you observe that the matrix is tilted to a higher degree, we recommend prolonging the standing time before conditioning. For quicker processing, we, recommend vortexing the Spin Columns upon receipt of the kit and storing them in 2 mL microcentrifuge tubes in upright position till used. Alternatively, we suggest using a swing-out rotor centrifuge with our Spin Column Adapter for Plate Centrifuges (product number: 050-011-024).
Poor performance in downstream experiments	**Tilted matrix** A tilted matrix 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 minutes before conditioning, see also tilted matrix in column, above, or section 2.2.

Poor performance in downstream experiments (continuation)	**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 rcf is not available, calculate the rpm see section 2.2. Always make sure to use the correct time mentioned in the protocol to avoid insufficient elution of RNA.
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. These pipettes should be cleaned thoroughly at regular intervals. It is also recommended to use filter tips for all pipetting steps involving samples. **Handling of samples** In general, work cautiously using sterile microbiological practices when working with RNA to avoid risk of contamination. Always wear gloves while handling reagents and RNA samples. Replace gloves regularly and keep tubes closed when possible. The use of pipette tips with filters is recommended.
Eluate is missing or volume to low	**Inappropriate handling of Spin Column** The Spin Column was closed during centrifugation, and vacuum was generated. See also tilted matrix in column, above, or section 2.2 for further instructions.

Eluate is missing or volume to low (continuation)	**Centrifuge settings** Most centrifuges offer the choice between rpm and *g*-force (rcf); if rcf is not available, calculate the rpm, see section 2.2. Always make sure to use 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 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 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 gDNA removal can only be achieved using the gDNA Removal Mix (product number 011-901-050 for 50 reactions and product number 011-901-250 for 250 reactions).

11. SYMBOLS

The following table describes the symbols that appear on the labeling of the EchoLUTION Cell Culture RNA products and in this user manual.

Table 6: ***EchoLUTION Cell Culture RNA Kit symbols.***

Symbols	Description
	Manufacturer
	Product number
	Batch code
	Contains sufficient for < n > reactions
	Temperature limitation
	Do not re-use
	Expiration date
	Consult instructions for use

WE ARE INTERESTED IN YOUR EXPERIENCE WITH BIOECHO PRODUCTS!

With questions or suggestions or for further troubleshooting, please contact us.

Visit our website and shop for further information, tutorials and application notes.

This user manual can be found in our shop on the corresponding product page.

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

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