EchoLUTION™ Cell Culture RNA Kit

384-well plate kits

USER MANUAL

REF

011-314-002

011-314-008

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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 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	1×10^6 cells
Purified nucleic acid	Total RNA
Elution volume	15 µL
Expected yields	Up to 10 µg (depends on amount of starting material)

2.1. *Single-step purification principle*

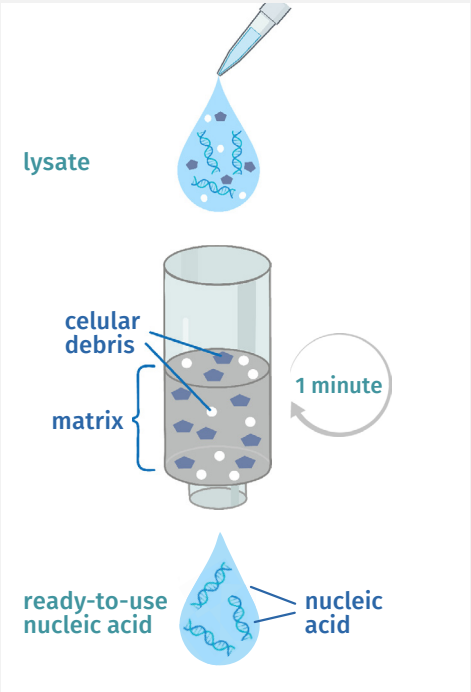
All BioEcho nucleic acid extraction kits are based on the EchoLUTION technology, which consists of tailored sample processing that includes lysis followed by single-step purification. The purification step works differently than conventional methods such as magnetic-bead and silica kits based on the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

The EchoLUTION purification matrix within the Purification Plate must be kept undamaged to avoid short-circuit currents. Short-circuit currents result in the introduction of lysis components into the eluate and inadequate purification, which can lead to inhibition in downstream analysis. Therefore, when applying the lysate to the well, **avoid touching the surface** of the filter matrix, and pipet **the sample very slowly (ideally dropwise)**.

To guarantee proper handling of the Purification Plate, be sure to use the recommended *g*-force centrifuge settings. Most centrifuges offer the choice between rpm and *g*-force (rcf); if not, calculate the rpm corresponding to the required *g*-force using the calculator in the link or the QR code below:

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

For support on suitable centrifuges, please contact us.

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 1×10^6 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 Kits, 384-well plate format***

Product number	011-314-002	011-314-008
Product name	EchoLUTION Cell Culture RNA Kit (2 × 384)	EchoLUTION Cell Culture RNA Kit (8 × 384)
Reactions	768	3,072
Lysis Buffer Cell RNA concentrate* (LB)	2 × 275 mg	4 × 550 mg
Lysis Solution Cell RNA* (LS)	2 × 20 mL	4 × 40 mL
Clearing Solution Cell RNA (CS)	7.5 mL	30 mL
Low-TE Buffer (T)	Blank only	Blank only
Purification Plate 384 Type 4	2	8
Elution Plate 384 Type 1	2	8
Adhesive Foil	2	8

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

3.2. Materials required but not provided

A. Conditioning Plate

The Conditioning Plate is necessary to remove the matrix storage buffer 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 060-006-008.

B. Multichannel reagent reservoir

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

C. Lysis Plate

The Lysis Plate necessary for mixing the Lysis Buffer Cell RNA (LB) with the sample is not included in the kit. BioEcho offers a suitable Lysis Plate for sale (product number 060-004-008). However, you can also use any 96-well plate with a capacity of at least 300 µL per well.

D. 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.

E. Adhesive Foils (depending on workflow)

Additional Adhesive Foils for incubation are needed, in case the ones included are used and discarded after lysis (product number 050-007-008 for 8 foils, or 050-007-050 for 50 foils). Typically, they can be opened for addition of Clearing Solution and re-sealed.

3.3. Laboratory equipment needed

A. Plate centrifuge

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

- Standardized Society for Biomolecular Screening (SBS) format
- Capable of at least 3,900 x *g*
- At least 5 cm clearance for plate holder height
- Swing-out rotor

B. Pipetting equipment

Pipetting can be performed using a liquid handling robot, a multi-channel pipette or a single-channel pipette. The use of wide-bore tips is recommended if mixing of lysate after addition of Clearing Solution is done via pipetting.

C. Thermal shaker for plates

One or more thermal shakers with agitation (up to 1,400 rpm), capable of heating up to 40 °C are used for the lysis and the optional genomic DNA (gDNA) removal step. For simultaneous lysis of 384 samples, a total capacity of four 96 microplates is needed. If just one thermal shaker is available, shut off heating after lysis step, as mentioned in the protocol.

D. Vortex mixer (optional)

A vortex mixer can be used for lysate mixture.

4. STORAGE AND STABILITY

4.1. Kit 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 one year.
- The Purification Plates need to be stored as described on the label (label facing up).

Before starting RNA extraction with the **EchoLUTION Cell Culture RNA Kit (REF: 011-314-002 and REF: 011-314-008)** 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, e.g. by vortex mixing, 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.

Table3: ***EchoLUTION Cell Culture RNA Kit safety information***

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

**Note: After addition of Lysis Solution Cell RNA (LS), concentration of TCEP is below critical levels and noprecautionary 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.
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 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, 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:

- 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 it to warm up to room temperature for minimum 10 minutes or until precipitation is dissolved.
- Pre-heat the thermal plate 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.*
- *Make sure the Lysis Buffer Cell RNA (LB) is prepared and warmed up to room temperature.*

1. Purification Plate preparation

- Detach first the lower and then the upper foil from the Purification Plate. Be sure to keep the plates in a horizontal position while removing the foils, because the wells contain liquid.
- Place the Purification Plate on top of the Conditioning Plate (not provided, product number 060-006-008).
- Centrifuge 1 minute at 1,000 x *g*, discard flow-through.
- Place the Purification Plate on top of the Elution Plate.
- Proceed directly with step 2.

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 1 × 10^6 cells in a 0.3 or 0.5 mL microplate (not provided, e.g., product number 060-004-008). Pellet cells by centrifugation at 500 x *g* for 5 minutes and remove the complete supernatant carefully.
- Add 50 µL Lysis Buffer Cell RNA (LB) to the prepared cells.
- Seal the plate with Adhesive Foil.
- 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.*
  - *Avoid disturbing the cell pellet while removing the media, as cell loss leads to reduced RNA yields.*
  - *When working with microplates below 0.3 mL volume the use of non-absorbent Sealing Foils (not provided, product number 050-008-050) is recommended.*
  - *The viscosity of the lysate increases with the cell number.*

b) Adherent cells

- Use up to 1 × 10^6 cells per reaction.
- If cells are grown in a 96-well cell culture plate, they can be lysed directly in the well. If cells are grown in other cell culture vessels, they must be trypsinized and pelleted by centrifugation before lysis.

Direct lysis:

- Completely aspirate the cell culture medium.
- Add 50 µL Lysis Buffer Cell RNA (LB) to the prepared cells.
- Seal the plate with Adhesive Foil.
- 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:

  - *Make sure that the Lysis Buffer Cell RNA (LB) covers the complete cell layer to ensure complete lysis.*

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 0.3 or 0.5 mL microplate (not provided, e.g., product number 060-004-008) and centrifuge at 500 x *g* for 5 minutes.
- Completely aspirate the supernatant.
- Add 50 µL Lysis Buffer Cell RNA (LB) to the prepared cells.
- Seal the plate with Adhesive Foil.
- 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).

  IMPORTANT NOTE:

  - *Ensure complete removal of the medium, as culture media can inhibit lysis, and thus 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 Lysis Plate, as cell loss leads to reduced RNA yields.*
  - *When working with microplates below 0.3 mL volume the use of non-absorbent Sealing Foils (not provided, product number 050-008-050) is recommended.*
  - *The viscosity of the lysate increases with the cell number.*

3. RNA Purification

- Open foil of the Lysis Plate and add 8 µL Clearing Solution (CS) to each sample. Mix, e.g., by pipetting or vortex mixing.
- Re-seal the plate with foil.

- Centrifuge for 5 minutes at maximum speed, up to 5,000 x *g*.
- Transfer 15 µL of the supernatant to the Purification Plate.

IMPORTANT NOTE:

- *If lysate and Clearing Solution are mixed by pipetting, we recommend the use of wide-bore tips.*
- *Pipet slowly, drop-by-drop, and vertically onto the middle of the wells to not destroy the matrix surface (use an 8-channel pipette or robot).*
- *Do not touch the matrix bed with the pipette tip during sample loading!*

- Centrifuge the loaded Purification Plate on top of Elution Plate for 1 minute 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. For storage of nucleic acids Sealing Foils (product number 050-008-050) can be purchased.*

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.

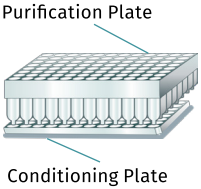
7.1. *Quick protocol EchoLUTION Cell Culture RNA Kit: 384-well plate formats*

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 plate centrifuge to 1,000 x *g*.

1. Purification Plate preparation

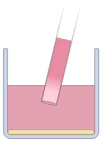


1000 x *g*
1 min

- 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.

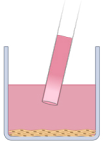
2. Sample preparation and lysis

Suspension cells



- Transfer cells to Lysis Plate.
- Spin and remove culture media.
- Add 50 µL LB.
- Incubate plate at 40 °C for 5 min at 1,400 rpm.

Adherent cells

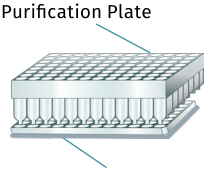


- Aspirate media.
- Lyse directly on plate or trypsinize (transfer to Lysis Plate).
- Add 50 µL LB to the cells.
- Incubate plate at 40 °C for 5 min at 1,400 rpm.

3. RNA purification

max. speed
5 min

- Add 8 µL CS.
- Mix.
- Seal plate and centrifuge for 5 min at max. speed.



1000 x *g*
1 min

- Transfer 15 µL supernatant. 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 WITH GDNA REMOVAL

A. Materials provided

Table 4: ***Content of the gDNA Removal Mix, for use with the EchoLUTION Cell Culture RNA Kit, 384-well plate format***

Product number	011-901-302	011-901-308
Product name	gDNA Removal Mix (2 × 384)	gDNA Removal Mix (8 × 384)
Solution A	8 × 300 µL	32 × 300 µL
Solution B	4 × 300 µL	16 × 300 µL

B. Materials required but not provided

- EchoLUTION Cell Culture RNA Kit (2 × 384 or 8 × 384)
- Conical centrifugation tubes for the preparation of master mixes.

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 purifying gDNA-free RNA from cultured cells using the EchoLUTION Cell Culture RNA Kit in combination with the gDNA Removal Mix: Purchase product number 011-901-302 for 2 × 384 or 011-901-308 for 8 × 384 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:

- Prepare the Lysis Buffer Cell RNA (LB) by adding the complete Lysis Solution Cell RNA (LS) to the Lysis Buffer Cell RNA (brown bottle, LB). 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.
- Lysis Solution Cell RNA (LS) or prepared Lysis Buffer Cell RNA (LB) may form precipitate 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.
- Carry out the complete RNA extraction at room temperature.

  IMPORTANT NOTE:

  - *Choose x g (RCF), NOT RPM, unless stated otherwise.*
  - *Make sure Lysis Buffer Cell RNA (LB) is prepared and warmed to room temperature.*
- Prepare a gDNA removal master mix for the number of samples with excess (according to the Table 2), mix gently and keep cold.

Number of samples	96 (incl. excess)	384 (incl. excess)	Your calculations
Suspension A	300 µL	1.2 mL	
Suspension B	150 µL	0.6 mL	
Final volume	**450 µL**	**1.8 mL**	

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

1. Sample preparation and lysis

a) Suspension cells

- Harvest up to 1 × 10^6 cells in a 0.3 or 0.5 mL microplate (not provided, e.g., product number 060-004-008). Pellet cells by centrifugation at 500 x *g* for 5 minutes and completely remove the supernatant carefully.
- Add 50 µL buffer Lysis Buffer Cell RNA (LB) to the prepared cells.
- Seal the plate with Adhesive Foil.
- Incubate the lysate 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 medium in the lysis can inhibit efficiency and, thereby, reduce RNA yield and quality.*
  - *Avoid disturbing the cell pellet when removing the media, as cell loss leads to reduced RNA yields.*
  - *When working with microplates that have well volumes below 0.3 mL, the use of non-absorbent Sealing Foils (not provided, product number 050-008-050) is recommended.*
  - *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.*

b) Adherent cells

- Use up to 1 × 10^6 cells per reaction.
- If cells are grown in a 96-well cell culture plate, cells 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 50 µL Lysis Buffer Cell RNA (LB) to the prepared cells.
- Seal plate with Adhesive Foil.
- 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:

  - *Make sure that Lysis Buffer Cell RNA (LB) completely covers the cell layer to ensure complete lysis.*
  - *If only one thermal shaker is available shut off heating after the lysis incubation to cool down thermal shaker for gDNA removal.*

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, and inactivate trypsin by adding medium (containing serum).
- Transfer cells to a 0.3 or 0.5 mL microplate (not provided, e.g., product number 060-004-008), and centrifuge at 500 x *g* for 5 minutes.
- Completely aspirate the supernatant.
- Add 50 µL Lysis Buffer Cell RNA (LB) to the prepared cells.
- Seal with Adhesive Foil.
- Incubate lysate 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.*
  - *Make sure to wash off trypsin, as trypsin carryover can reduce RNA yield and quality.*
  - *Transfer all cells to the Lysis Plate, as cell loss leads to reduced RNA yields.*
  - *When working with microplates with well volumes of less than 0.3 mL, the use of non-absorbent Sealing Foils (not provided, product number 050-008-050) is recommended.*

- *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 foil of the lysis plate, add 8 µL Clearing Solution Cell RNA (CS) to each sample and mix thoroughly, e.g., by pipetting or vortex mixing.
- Re-seal the plate with foil.
- Centrifuge for 5 minutes at maximum speed, up to 5,000 x *g*.
- Open foil, add 4 µL gDNA removal master mix to the lysates (supernatant), and re-seal.
- Incubate pelleted lysate in a thermal shaker with constant shaking at 300 rpm for 10 minutes at room temperature.

NOTE:

- *If lysate and Clearing Solution are mixed by pipetting, we recommend the use of wide-bore tips.*
- *When adding the gDNA removal master mix to the samples, make sure to not touch the pellet.*
- *Make sure to not shake the pelleted lysate with more than 300 rpm.*
- *If only one thermal shaker is available, you can turn off the heat after the lysis incubation step and incubate the plate in the warm shaker, up to 40°C.*
- *During incubation, proceed with step 3.*

3. Purification Plate preparation

- Detach first the lower and then the upper foil from the Purification Plate. Please keep the plates in a horizontal position while removing the foils, as the wells contain liquid.
- Place the Purification Plate on top of the Conditioning Plate (not provided, product number 060-006-008).
- Centrifuge 1 minute at 1,000 x *g*, and discard flow-through.
- Place the Purification Plate on top of the Elution Plate.

NOTE:

- *The centrifuge rotor should be able to hold plate stacks of at least 5 cm height. Conditioning Plates can be reused.*

4. *RNA Purification*

- Transfer 15 µL of the lysate (supernatant) to the Purification Plate.

  IMPORTANT NOTE:

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

- Centrifuge the loaded Purification Plate on top of the Elution Plate for 1 minute 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 use of Sealing Foils (product number 050-008-050) is recommended.*

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.

8.2. Quick protocol EchoLUTION Cell Culture RNA Kit: 384-well plate formats with gDNA Removal Mix

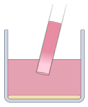
IMPORTANT NOTE: *Please use the quick protocol only after you have read and understood the complete user manual.*

Preparation before starting

- Prepare the Lysis Buffer Cell RNA (LB).
- Pre-heat the thermal shaker to 40 °C.
- Set the plate centrifuge to 1,000 x *g*.
- Prepare a gDNA removal master mix.

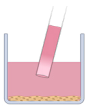
1. Sample preparation and lysis

Suspension cells



- Transfer cells to Lysis Plate.
- Spin and remove culture media.

Adherent cells



- Aspirate media.
- Lyse directly on plate or trypsinize (transfer to Lysis Plate).

- Add 50 µL LB to the cells.
- Incubate sealed plate at 40 °C for 5 min at 1,400 rpm.

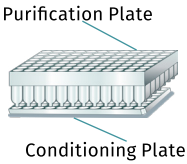
2. gDNA removal

max. speed 5 min

- Add 8 µL CS and **mix**.
- Seal plate and centrifuge for 5 min at max. speed.
- Add 4 µL of DNA removal master mix and seal plate.
- Incubate 10 min at room temperature and 300 rpm.

3. Purification Plate preparation

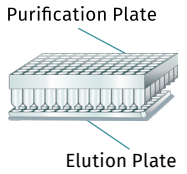
1000 x *g* 1 min



- 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.

4. RNA purification

1000 x *g* 1 min



- Transfer 15 µL 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
RNA yield and concentration is low	**The 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 1 × 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, reduce 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 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.

RNA yield and concentration is low (continuation)	**Loading of the 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 each well to not destroy the matrix surface. Do not touch the matrix surface 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 section 2.2. Always make sure to use to 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 and not RNase free water in measurements. **Sample input** Always use up to 1×10^6 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. 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.

Degraded RNA (continuation)	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 1×10^6 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. **Optimal procedure not used** In general, the purification matrix eliminates most gDNA. However, certain sensitive RNA downstream applications may require further gDNA removal. Use the gDNA Removal Mix, product number 011-901-302 and 011-901-308, see section 8.
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 matrix
A tilted matrix bed can lead to inappropriate sample flow through and therefore insufficient time of interaction with the matrix surface, which can lead to poor extraction performance. If you 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
Most centrifuges offer the choice between rpm and *g*-force (rcf); if not, 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	**Sealing of Purification Plate** The use of non-air-permeable foils creates 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 050-007-008 for 8 foils and 050-007-050 for 50 foils). **Centrifuge settings** Low centrifugation settings can lead to inadequate elution. Most centrifuges offer the choice between rpm and *g*-force (rcf); if not, calculate the rpm from the recommended rcf, 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 011-901-002 and 011-901-008, see section 8).

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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