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QIAmini RNA Tissue/Cells Kit

Instructions for Use

For automated purification of total RNA including small RNAs from tissue samples and cells using QIAmini

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Kit Contents

QIAmini RNA Tissue/Cells Kit	(48)
Catalog no.	591105
Number of reactions	48
Buffer RLT*	45 mL
Proteinase K	1.4 mL
RNase-free DNase I (lyophilized)	1500 units
RNase-free water	15 mL
QIAmini RNA Tissue/Cells Cartridges*	48
Rod Covers	12
Quick-Start Protocol	1

* Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. See the Safety Information section.

Shipping and Storage

The QIAmini RNA Tissue/Cells Kit is shipped at ambient temperature. Upon receipt, store DNase I at 2–8°C. Store all other kit components dry at room temperature (15–25°C).

When stored properly, buffers and reagent cartridges are stable until the expiration date on the kit label.

Intended Use

The QIAmini RNA Tissue/Cells Kit is intended for research use only. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF at www.qiagen.com/safety, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION

Do not use bleach, or acidic solutions directly to the sample preparation waste.



Buffer RLT and buffers in the QIAmini RNA Tissue/Cells Cartridges contain chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

If liquid containing potentially infectious agents is spilled on QIAmini, refer to the instrument user manual for decontamination instructions.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAmini RNA Tissue/Cells Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAmini RNA Tissue/Cells Kit enables automated purification of RNA and miRNA from cells and tissue samples using QIAmini. For detailed description of QIAmini, refer to the *QIAmini User Manual* (www.qiagen.com/HB-3799) and the *QIAmini Suite User Guide* (www.qiagen.com/HB-3800). The QIAmini RNA Tissue/Cells Kit enables fully automated processing of samples after lysis, significantly reducing manual steps and hands-on time for greater convenience.

Principle and procedure

The QIAmini RNA Tissue/Cells Kit combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles in ready-to-use, pre-filled cartridges. The purification procedure is designed to ensure simple, safe, and reproducible processing of tissue and cell samples. The purification procedure comprises four steps: sample preparation and lysis, binding, washing, and elution.

The QIAmini RNA Tissue/Cells application provides a fully automated protocol for the purification of RNA from cells and tissue samples. Before loading the cartridges onto QIAmini, the procedure begins with a manual lysis step using Buffer RLT, which protects RNA molecules. This step is followed by Proteinase K digestion under optimized conditions to ensure complete lysis of even difficult-to-lyse tissues and efficient release of RNA. Following the addition of the lysate to the cartridge, the cartridge is loaded onto QIAmini. During automated purification, RNA binds to magnetic particles.

While the nucleic acids remain bound to the magnetic particles, contaminants are efficiently washed away during a sequence of wash steps. The standard QIAmini RNA Tissue/Cells workflow includes a DNase digestion step using RNase-free DNase, which is performed within the cartridge during the automated purification process. Alternatively, the DNase digestion step can be omitted to recover total nucleic acids (RNA and DNA) in a single eluate. In the final step, highly purified RNA is eluted in RNase-free water.

The purified nucleic acid can be either used immediately in downstream applications or stored for future use.

Automation

QIAmini can perform all steps following lysis and disruption of the sample. This automation is based on magnetic particle technology and includes nucleic acid binding, optional enzymatic digestion, washing, and elution. Up to eight samples can be processed in a single run.

Magnetic-particle technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. Total nucleic acid or RNA-only is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are subsequently separated from the lysates using a magnet. Additional washing steps remove any residual contaminants. Finally, the nucleic acids are efficiently eluted.

Equipment and Reagents Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier. These are available online in convenient and compact PDF at www.qiagen.com/safety where you can find, view, and print the SDS kit for each QIAGEN kit and kit component.

The following materials are not included in the kit and are to be supplied by the user:

All protocols

- Pipettes and sterile, RNase-free pipette tips
- Disposable gloves
- 1.5 mL tubes
- 2 mL tubes

For cells

- **Optional:** Vortexer

For tissue samples

- TissueLyser III or a similar device
- Stainless Steel Beads, 5 mm (cat. no. 69989)

Important Notes

Determining the correct amount of starting material — cells

The minimum amount of starting material is approximately 100 cells. The maximum amount depends on the RNA content of the cell type but should not exceed 5×10^6 cells.

RNA content can vary greatly between cell types. The following examples illustrate how to determine the maximum amount of starting material:

- COS cells have high RNA content (approximately 35 µg RNA per 1×10^6 cells).
- HeLa cells have average RNA content (approximately 15 µg RNA per 1×10^6 cells).
- NIH/3T3 cells have low RNA content (approximately 10 µg RNA per 1×10^6 cells).

In general, we recommend starting with no more than 5×10^6 cells. Depending on RNA yield and purity, it may be possible to increase the cell number in subsequent preparations.

Counting cells is the most accurate way to quantify the amount of starting material. As a guide, the number of HeLa cells obtained in various culture vessels after confluent growth is shown in Table 1.

Table 1. Growth area and number of HeLa cells in various culture vessels

Cell-culture vessel	Growth area (cm ²)*	Number of cells†
Multiple plates		
96 wells	0.32–0.6	4–5 × 10 ⁴
48 wells	1	1 × 10 ⁵
24 wells	2	2.5 × 10 ⁵
12 wells	4	5 × 10 ⁵
6 wells	9.5	1 × 10 ⁶
Dishes		
35 mm	8	1 × 10 ⁶
60 mm	21	2.5 × 10 ⁶
100 mm	56	7 × 10 ⁶
145–150 mm	145	2 × 10 ⁷

* Per well, if multiwell plates are used; values may vary slightly depending on the supplier.

† Cell numbers are given for HeLa cells (average length, approx. 15 µm), assuming confluent growth. Cell numbers vary among animal cell types, which typically range from 10 to 30 µm in length.

Determining the correct amount of starting material — tissue

The maximum amount of tissue that can be processed depends on the RNA content of the tissue. In general, a maximum of 50 mg of tissue can be processed using the QIAmini RNA Tissue/Cells Kit. If no information about the nature of the starting material is available, we recommend starting with no more than 10 mg of tissue.

Weighing tissue is the most accurate way to quantify the amount of starting material. However, the following guideline may be used: a 3 mm cube (volume = 27 mm³) of most animal tissues weighs approximately 25–35 mg.

Handling and storage of starting material

RNA is not protected after harvesting until the sample is treated with RNAprotect® Cell Reagent (cultured cells only) or RNAprotect Tissue Reagent (animal tissues only), flash-frozen, or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. Otherwise, unwanted changes in the gene expression profile may occur. It is therefore important that samples are either processed as soon as they are harvested, immediately immersed in RNAprotect Cell Reagent or RNAprotect Tissue Reagent, or immediately frozen in liquid nitrogen and stored at -90°C to -65°C . Animal cells can be pelleted and stored at -90°C to -65°C until required for RNA purification.

An alternative to RNAprotect Tissue Reagent is Allprotect® Tissue Reagent, which provides immediate stabilization of DNA, RNA, and protein in tissue samples at room temperature. Because tissues stabilized with RNAprotect Tissue Reagent or Allprotect Tissue Reagent are partially dehydrated, a lower amount of starting material should be used.

Procedures for harvesting and RNA protection should be carried out as quickly as possible. Frozen samples must not be allowed to thaw during handling or weighing. After disruption and homogenization in Buffer RLT, samples can be stored at -90°C to -65°C for several months.

Disruption and homogenization of starting material

Efficient disruption and homogenization of the starting material are required for all RNA purification procedures. Disruption and homogenization are two distinct steps:

Disruption

Complete disruption of the plasma membranes of cells and organelles is essential to release all RNA contained in the sample. Different sample types require different methods to achieve complete disruption. Incomplete disruption significantly reduces RNA yields.

Homogenization

Homogenization is necessary to reduce the viscosity of lysates generated during disruption. This process shears high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. With the QIAmini RNA Tissue/Cells Kit, up to 5×10^6 cells can typically be processed by vortexing. If more cells must be processed, additional homogenization is required (see Table 2). Incomplete homogenization results in inefficient binding of RNA to magnetic particles, significantly reducing RNA yields.

Some disruption methods simultaneously disrupt and homogenize the sample, whereas others require an additional homogenization step. Table 2 provides an overview of different disruption and homogenization methods. This information can be used as a guide to select the appropriate method for the starting material.

Table 2. Guide to disruption and homogenization methods for samples

Sample	Disruption method	Homogenization method	Comments
Animal cells	Addition of lysis buffer	TissueRuptor® II or QIAshredder homogenizer or syringe and needle or vortexing ($\leq 3 \times 10^6$ cells)	If processing $\leq 1 \times 10^6$ cells, lysate can be homogenized by vortexing.
Animal tissues	TissueLyser III or TissueLyser LT	TissueLyser III or TissueLyser LT	The TissueLyser III and TissueLyser LT give results comparable to using a rotor–stator homogenizer.
	TissueRuptor II	TissueRuptor II	Simultaneously disrupts and homogenizes
	Mortar and pestle	QIAshredder homogenizer or syringe and needle	The TissueRuptor II usually gives higher yields than mortar and pestle.

Disruption and homogenization using the TissueLyser III or TissueLyser LT

In bead milling, cells and tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur through the shearing and crushing action of the beads as they collide with the cells. Disruption efficiency is influenced by the following factors:

- Size and composition of beads
- Ratio of buffer to beads
- Amount of starting material
- Speed and configuration of the TissueLyser III or TissueLyser LT
- Disintegration time

Stainless steel beads with a diameter of 3–7 mm are optimal for use with animal tissues. All other disruption parameters must be determined empirically for each application. For other bead mills, refer to the suppliers’ guidelines for further details.

Preparation of DNase I stock solution

Prepare the DNase I stock solution by dissolving the lyophilized DNase I (1500 Kunitz units) in 550 μL of RNase-free water. In some cases, the DNase I vial may appear empty because the lyophilized enzyme adheres to the septum. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe, and mix gently by inverting the vial. Do not vortex.

Insoluble material may remain after dissolving DNase I; this does not affect DNase I performance. Owing to the production process, insoluble material may be present in the lyophilized enzyme. However, rigorous quality control tests are performed to ensure consistent DNase I activity from lot to lot.

Note: Do not vortex reconstituted DNase I, as it is sensitive to physical denaturation. Mix only by gently inverting the vial.

For long-term storage, remove the DNase I stock solution from the vial, divide it into single-use aliquots, and store at -30°C to -15°C for up to 9 months. Thawed aliquots can be stored at $2-8^{\circ}\text{C}$ for up to 4 weeks. Do not refreeze aliquots after thawing.

Before starting a run that includes a DNase digestion step, add 10 μL of reconstituted DNase to well 3 of each cartridge as described in the protocol.

Preparing samples

The purification procedure is for the extraction of total RNA including miRNA from up to 5×10^6 cells and up to 50 mg fresh/frozen tissue or 25 mg stabilized tissue. If information about the nature of starting material is not available, starting with no more than 10 mg of tissue is recommended.

Elution volumes and eluate handling

In the final step of the purification procedure, the RNA is eluted in RNase-free water. Before starting the run, add the desired volume of RNase-free water to well 5 of each cartridge, as described in the protocol. RNase-free water is supplied separately and is not pre-filled in the cartridge, allowing the elution volume to be adjusted according to the application. The protocol is optimized for an elution input volume of 100 μL , which results in an eluate volume of approximately 80 μL . As with all magnetic particle-based purification methods, approximately 20 μL of elution buffer remains associated with the magnetic particles (dead volume). The protocol is optimized for a target eluate volume of 50–200 μL and requires the addition of 1 μL elution buffer per 1 μL magnetic particles to counteract the dead volume* of the magnetic particles. The default setting for the elution input volume is 100 μL , which results in an elution volume of approximately 80 μL .

Storing RNA

If RNA is used directly in downstream applications, it can be kept on ice. For longer storage, RNA should be stored at -20°C or -70°C .

Working with QIAmini

The main features of QIAmini include the following:

- Purification of high-quality nucleic acids from a wide variety of sample types
- Flexible throughput, allowing processing of up to eight samples per run
- Simple, low-complexity automation of magnetic, particle-based bind–wash–elute workflows
- Pre-filled reagent cartridges enabling convenient load-and-go operation

*Dead volume is the amount of liquid that cannot be recovered due to the inherent features of the magnetic particles.

- Compact footprint, designed to fit on any laboratory bench and allow rapid implementation
- User-friendly operation, making automated nucleic acid purification accessible even for occasional users
- Browser-based application for protocol management
- UV decontamination to help reduce sample carryover from run-to-run and allow pathogen decontamination on worktable surface

Note: UV decontamination helps reduce possible pathogen contamination on QIAmini worktable surfaces. The efficiency of inactivation must be determined for each specific organism and depends on factors such as sample type and contaminant load. QIAGEN cannot guarantee complete eradication of specific pathogens.

Protocol: QIAmini RNA Tissue/Cells Kit

Important notes before starting

- A list of all imported protocols is available in the Run menu list view.
- For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* (www.qiagen.com/HB-3870), available for download separately.
- Download the appropriate protocol file from www.qiagen.com/QIAmini or from QIAmini Suite (www.qiagen.com/QIAminiSuite), and transfer to your QIAmini instrument.
- Buffer RLT contains guanidine salt and is therefore not compatible with disinfecting reagents containing bleach.
- If necessary, redissolve any precipitate in Buffer RLT by warming.
- Equilibrate buffers to room temperature.
- Up to eight cartridges can be processed in one run.
- Unless stated otherwise, perform all steps at room temperature. Work quickly.
- If purifying RNA from cell lines rich in RNases or from tissue, we recommend adding either β -mercaptoethanol (β -ME) or 2 M dithiothreitol (DTT) to Buffer RLT before use (10 μ L β -ME or 20 μ L DTT per 1 mL Buffer RLT). Buffer RLT containing DTT or β -ME can be stored at room temperature for up to 1 month.
- In the following procedure, text marked with ● denotes RNA purification from cells and text marked with ▲ denotes RNA purification from tissue samples.

- **Optional:** If DNA digest is required, prepare DNase I stock enzyme by adding 550 μL RNase-free water to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in appropriate portions and store at -30°C to -15°C for long-term storage. Avoid freezing–thawing more than three times. DNase I is sensitive to physical denaturation; do not vortex resuspended DNase I.

Procedure

Sample preparation

1. • Harvest up to 5×10^6 cells as a cell pellet or, for cells grown in a monolayer, aspirate and discard the cell culture medium from the cell culture vessel (up to 10 cm in diameter). Add 300 μL Buffer RLT to the pellet or directly to the cell culture vessel and homogenize. Transfer the lysate to a 2 mL tube (not provided).

▲ Add 300 μL Buffer RLT to up to 50 mg frozen tissue or 25 mg stabilized tissue, then disrupt and homogenize. Transfer the lysate to a 2 mL tube (not provided).

Proceed with step 2.

Note: See Table 2 for disruption and homogenization methods.

2. Prepare a master mix ($x + 10\%$ samples) by mixing 75 μL RNase-free water and 25 μL Proteinase K per sample. Mix thoroughly. Add 100 μL of the Proteinase K mix to each sample, mix thoroughly, and incubate for 10 min at room temperature. In the meantime, prepare the cartridge as described in the next section.

Cartridge preparation

3. Invert the pre-filled cartridge(s) to resuspend magnetic beads. Flick down to remove reagents and bead suspension from under the foil.
4. Place the cartridge(s) into the cartridge frame. Make sure to keep the pull tab accessible. Ensure that the cartridge(s) are fully inserted into the cartridge frame.
5. **Important:** Carefully remove the sealing foil. Avoid splashing of reagents.
6. Transfer RNase-free water into well 5 of the cartridge for elution with the respective volume according to the selected protocol.

Optional: If doing a DNase digestion, add 10 µL reconstituted DNase to well 3.

7. Add 400 µL lysate to well 1 of the cartridge.

Procedure on QIAmini

1. Turn on QIAmini.
2. Select the desired protocol.

Note: A list of all imported protocols is available in the Run menu list view.

Note: For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* ([www.qiagen.com/HB- 3870](http://www.qiagen.com/HB-3870)), available for download separately.

3. Open the instrument door. Load the cartridge frame holding the cartridge(s) into the instrument.

Note: Ensure that the cartridge frame is placed in correct orientation. Press the cartridges down to fully engage with the heater block.

4. Slide rod cover(s) into the rod cover holder.

Note: Press the clip down to allow full insertion of the rod cover holder.

5. Close the instrument door, press , and confirm that consumables have been installed correctly to initiate the protocol run.
6. The display shows "End of run" when the run is completed. Select **Confirm** and press .
7. Open the instrument door. Remove and discard the rod cover(s). Then remove the cartridge frame holding the cartridge(s) and transfer the purified nucleic acids from well 5 of each cartridge into a new reaction tube (e.g., 1.5 mL tube). Discard the cartridge(s).

Recommended: Clean the instrument and perform a UV decontamination of the instrument chamber.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx (for contact information, visit support.qiagen.com).

Comments and suggestions	
General handling	
Error message in instrument	Refer to the <i>QIAmini User Manual</i> (www.qiagen.com/HB-3799).
Loss of reagents from the cartridge when removing the seal	After inverting the reagent cartridges to resuspend the magnetic particles, make sure to flick down the cartridges to deposit the reagents at the bottom of the wells.
RNA degraded	
Inappropriate handling of starting material	Ensure that tissue samples are properly stabilized and stored in RNAprotect Tissue Reagent. For frozen cell pellets or frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at -90°C to -65°C. Perform the procedure quickly, especially the first few steps. See "Handling and storage of starting material".
RNase contamination	Although all buffers in the QIAmini RNA Tissue/Cells Kit have been tested and are guaranteed to be RNase free, RNases can be introduced during use. Be certain not to introduce any RNases during the procedure or during subsequent handling.
DNA contamination in downstream experiments	
Cell number too high	For some cell types, the efficiency of DNA removal may be reduced when processing very high cell numbers (containing more than 20 µg gDNA). If the eluted RNA contains substantial DNA contamination, try processing a smaller number of cells or perform a second DNase digestion of the eluted RNA followed by RNA cleanup.
Tissue has high DNA content	For certain tissues with extremely high DNA content (e.g., thymus), DNA may not be completely removed. Try using smaller samples (containing less than 20 µg gDNA) or perform a second DNase I digestion of the eluted RNA followed by RNA cleanup.

Comments and suggestions

Beads in eluate

Too much input material used	Adhere to the instructions regarding sample material, and carry out initial tests with lower amounts. Too much material may increase viscosity of the sample that can impact bead handling throughout the protocol.
Incomplete disruption or homogenization	When using high amounts of sample material, more vigorous lysis such as the listed mechanical lysis options may be required to help with sample disruption and homogenization.

Ordering Information

Product	Contents	Cat. no.
QIAmini RNA Tissue/Cells Kit (48)	For automated purification of total RNA, including small RNAs, from animal tissues and cultured cells using QIAmini	591105
Related products		
QIAmini	Benchtop instrument for automated isolation of nucleic acids; includes 1 year warranty on parts and labor	Varies*
QIAmini Rod Covers (50)	Disposable rod covers for use with QIAmini; 50 rod covers	590025
TissueLyser III	Bead mill, 100–120/220–240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 × 24 or TissueLyser Adapter Set 2 × 96	9003240
TissueLyser Adapter Set 2 × 24	Two sets of adapter plates and two racks for use with 2 × 24 samples in 2 mL microcentrifuge tubes on the TissueLyser III	69982
Plate Adapter Set	2 sets of adapter plates for use with two 96- well plates on the TissueLyser III, compatible with the PowerBead Pro Plates and 2 mL Tube Holder Set	11990
2 mL Tube Holder Set	For sample homogenization of up to 2 × 48 samples in 2 mL bead tubes on the TissueLyser III	11993
Stainless Steel Beads, 5 mm (200)	200 stainless steel beads (5 mm diameter), suitable for use with TissueLyser systems	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
Buffer RLT	220 mL RNeasy Lysis Buffer	79216
RNAprotect Cell Reagent (250 mL)	250 mL RNAprotect Cell Reagent	76526
RNAprotect Tissue Reagent (250 mL)	250 mL RNAprotect Tissue Reagent for stabilization of RNA	76106
Allprotect Tissue Reagent (100 mL)	100 mL Allprotect Tissue Reagent for stabilization of DNA, RNA, and protein in tissue samples	76405

* Contact your QIAGEN sales team or local distributor to determine the instrument available in your country.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit instructions for use or user manual. QIAGEN kit instructions for use and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Service or your local distributor.

Document Revision History

Date	Description
08/2026	Initial release

Limited License Agreement for QIAmini RNA Tissue/Cells Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this Instructions for Use and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this panel with any components not included within this panel except as described in the protocols provided with the product, this Instructions for Use, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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