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RNeasy® UCP Micro Kit Handbook

For purification of up to 45 µg total RNA from small or low biomass samples

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy UCP Micro Kit **Catalog no.** **Number of reactions**	**(50)** **73934** **50**
RNeasy UCP MinElute® Spin Columns (each in a 2 ml Collection Tube)	50
Collection Tubes (1.5 ml)	50
Collection Tubes (2 ml)	50
Buffer RULT*	45 ml
Buffer RUWT*† (concentrate)	15 ml
Buffer RUPE† (concentrate)	11 ml
Ultra-clean water	3 x 1 ml
RNase-Free DNase Set	
RNase-Free DNase I (lyophilized)	1500 units
Buffer RDD	2 x 2 ml
RNase-Free Water	1.5 ml
Quick-Start Protocol	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 5 for Safety Information.

† Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Shipping and Storage

The RNeasy UCP Micro Kit is shipped at ambient temperature. Store the RNeasy UCP MinElute spin columns and the RNase-Free DNase Set (i.e., the box containing RNase-free DNase, Buffer RDD, and RNase-free water) immediately upon receipt at 2–8°C. Store the remaining components of the kit dry at room temperature (15–25°C). All kit components are stable for at least 9 months under these conditions.

Intended Use

The RNeasy UCP Micro Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

The QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of the QIAcube Connect.

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 format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION 	DO NOT add bleach or acidic solutions directly to the sample preparation waste

Buffer RULT contains guanidine thiocyanate, and Buffer RUWT contains a small amount of guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of RNeasy UCP Micro Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

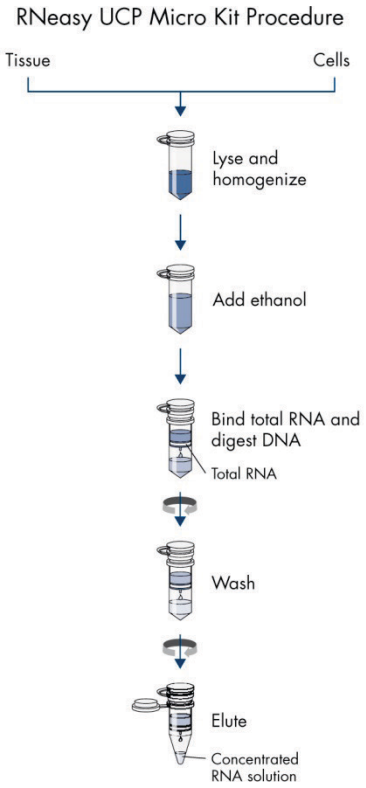
The RNeasy UCP Micro Kit is designed to purify RNA (maximum 45 µg) from small amounts of tissue or cells. The RNeasy UCP Micro Kit is manufactured using ultra-clean production (UCP) to ensure minimal residual contamination with exogenous nucleic acids.

QIAGEN provides a wide range of other kits for purification of total RNA from different sample materials: for details, visit **www.qiagen.com/RNA-Selection-Tools**.

Principle and procedure

RNeasy UCP Micro technology combines the selective binding properties of an ultra-clean silica-based membrane with the speed of microspin technology. Ultra-clean guanidine-thiocyanate-containing RULT lysis buffer and ethanol are added to the sample to create conditions that promote selective binding of RNA to the RNeasy UCP MinElute membrane. The sample is then applied to the RNeasy UCP MinElute spin column and RNA binds to the silica membrane. Traces of DNA that may copurify are removed by DNase treatment on the RNeasy UCP MinElute spin column. DNase and any contaminants are washed away with ultra-clean wash buffers RUWT and RUPE, and high-quality RNA is eluted in ultra-clean water (see RNeasy UCP Micro Kit Procedure flowchart, page 7). With the RNeasy UCP Micro procedure, all RNA molecules longer than 200 nucleotides are purified. The procedure enriches for mRNA because most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA and tRNAs, which together make up 15–20% of total RNA) are selectively excluded.

In this handbook, different protocols are provided for cells and tissue. The protocols differ primarily in the lysis and homogenization of the sample and in the adjustment of the conditions for binding RNA to the RNeasy UCP MinElute membrane. Once the sample is bound to the membrane, the protocols are similar.


RNeasy UCP Micro Kit Procedure
Tissue
Cells
Lyse and homogenize
Add ethanol
Bind total RNA and digest DNA
Total RNA
Wash
Elute
Concentrated RNA solution

Equipment and Reagents to Be 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.

For all protocols

- 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M) or, alternatively, 2 M dithiothreitol (DTT) in water
- Ethanol (70%, 80%, and 96–100%)*
- Sterile, RNase-free pipet tips
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer
- Disposable gloves
- Reagent for RNA stabilization (see page 12):
- For cell samples: RNAprotect® Cell Reagent or liquid nitrogen
- For tissue samples: RNAprotect Tissue Reagent (stabilizes RNA only), Allprotect® Tissue Reagent (stabilizes DNA, RNA, and protein), or liquid nitrogen
- Equipment for sample disruption and homogenization (see page 13).

Depending on the method chosen, one or more of the following are required:

- Trypsin and PBS
- Blunt-ended needle and syringe
- Mortar and pestle
- TissueRuptor® II with TissueRuptor Disposable Probes
- TissueLyser II

* Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone.

Automated purification of RNA on QIAcube instruments

Purification of RNA can be fully automated on the QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the RNeasy UCP Micro Kit for purification of high-quality RNA.

The QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

QIAcube Connect.

Important Notes

Determining the amount of starting material

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. The maximum amount that can be used is determined by:

- The type of sample and its RNA content
- The volume of Buffer RULT required for efficient lysis
- The RNA binding capacity of the RNeasy UCP MinElute spin column

When processing samples containing high amounts of RNA, less than the maximum amount of starting material shown in Table 1 should be used, so that the RNA binding capacity of the RNeasy UCP MinElute spin column is not exceeded.

When processing samples containing an average or low amount of RNA, use the maximum amount of starting material as shown in Table 1. However, even though the RNA binding capacity of the RNeasy UCP MinElute spin column is not reached, the maximum amount of starting material must not be exceeded; otherwise, lysis will be incomplete and cellular debris may interfere with the binding of RNA to the RNeasy UCP spin-column membrane, resulting in lower RNA yield and purity. More information on using the correct amount of starting material is given in each protocol. Table 2 shows typical RNA yields from various cells and tissues.

Note: If the binding capacity of the RNeasy UCP MinElute spin column is exceeded, RNA yields will not be consistent and may be reduced. If lysis of the starting material is incomplete, RNA yields will be lower than expected, even if the binding capacity of the RNeasy UCP MinElute spin column is not exceeded.

Table 1. RNeasy UCP MinElute spin column specifications

Product details	
Maximum binding capacity	45 µg RNA
Maximum loading volume	700 µl
RNA size distribution RNA	>200 nucleotides
Minimum elution volume	10–20 µl
Maximum amount of starting materials	
Animal and human cells	5×10^5
Animal and human tissues	5 mg

Table 2. Typical yields of total RNA with the RNeasy UCP Micro Kit

Cell type	RNA,* µg per 5×10^5 cells
NIH/3T3	5
HeLa	7.5
COS-7	17.5
LMH	6
Huh	7.5
Mouse/rat tissues (5 mg)	
Embryo (13 day)	10
Brain	4
Heart†	5
Kidney	15
Liver	15
Spleen	15
Thymus	20
Lung	5

* Amounts can vary due to factors, such as species, developmental stage, and growth conditions. Since the RNeasy UCP Micro procedure enriches for mRNA and other RNA species >200 nucleotides, the total RNA yield does not include 5S rRNA, tRNA, and other low-molecular-weight RNAs, which make up 15–20% of total cellular RNA.

Handling and storing starting material

Cells

After harvesting, cells should be immediately lysed in Buffer RULT to prevent unwanted changes in the gene expression profile. This highly denaturing lysis buffer inactivates RNases and other proteins to prevent RNA degradation as well as downregulation or upregulation of transcripts.

If the cells are to be shipped to another lab for RNA purification, they should be pelleted, frozen in liquid nitrogen, and transported on dry ice. Alternatively, the cells can be mixed with RNAprotect Cell Reagent at room temperature and then shipped at ambient temperature.

Tissues

RNA in harvested tissue is not protected until the sample is treated with RNAprotect Tissue Reagent, flash-frozen, or disrupted and homogenized in the presence of an RNase-inhibiting or -denaturing reagent; otherwise, unwanted changes in the gene expression profile will occur. It is important that tissue samples are immediately frozen in liquid nitrogen and stored at –70°C or immediately immersed in RNAprotect Tissue Reagent. An alternative to RNAprotect Tissue Reagent is the Allprotect Tissue Reagent, which provides immediate stabilization of DNA, RNA, and protein in tissue samples at room temperature.

The procedure for tissue harvesting and RNA protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or weighing. After disruption and homogenization in Buffer RULT (lysis buffer), samples can be stored at –70°C for several months.

Disrupting and homogenizing starting material

Efficient disruption and homogenization of the starting material is an absolute requirement for all total RNA purification procedures. Disruption and homogenization involve two distinct steps:

- **Disruption**: Complete disruption of cell plasma membranes and organelles is absolutely required to release all the RNA contained in the sample. Different samples require different methods to achieve complete disruption. Incomplete disruption results in significantly reduced RNA yields.
- **Homogenization**: Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Homogenization shears the high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of RNA to the RNeasy UCP MinElute spin-column membrane and therefore significantly reduced RNA yields.

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step. Table 3 gives an overview of different disruption and homogenization methods and is followed by a detailed description of each method.

Table 3. Disruption and homogenization methods

Sample	Disruption method	Homogenization method
Cells	Addition of lysis buffer	TissueRuptor II or syringe and needle*
Tissues	TissueRuptor II† TissueLyser II‡ Mortar and pestle§	TissueLyser II‡ or syringe and needle*

* If processing ≤1 x 10^5 cells, the lysate can be homogenized by vortexing.

† Simultaneously disrupts and homogenizes individual samples.

‡ Simultaneously disrupts and homogenizes up to 192 samples in parallel. Results are comparable to those obtained using the TissueRuptor or other rotor–stator homogenizers.

§ The TissueRuptor and TissueLyser usually give higher RNA yields than mortar and pestle.

Disruption and homogenization using the TissueRuptor II

The TissueRuptor II is a rotor–stator homogenizer that thoroughly disrupts and simultaneously homogenizes single tissue samples in the presence of lysis buffer in 15–90 seconds, depending on the toughness and size of the sample. The TissueRuptor II can also be used to homogenize cell lysates. The blade of the TissueRuptor II rotates at a very high speed, causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. For guidelines on using the TissueRuptor II, refer to the *TissueRuptor II Handbook*. For other rotor–stator homogenizers, refer to the suppliers' guidelines.

Disruption and homogenization using the TissueLyser II

In bead milling, tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the cells. The TissueLyser II disrupts and homogenizes up to 48 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 24, which holds 48 x 2 ml microcentrifuge tubes containing stainless steel beads of 5 mm mean diameter. The TissueLyser II can also disrupt and homogenize up to 192 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 96, which holds 192 x 1.2 ml microtubes containing stainless steel beads of 5 mm mean diameter. For guidelines on using the TissueLyser II, refer to the *TissueLyser Handbook*. For other bead mills, refer to the suppliers' guidelines.

Note: Tungsten carbide beads react with Buffer RULT and must not be used to disrupt and homogenize tissues.

Disruption using a mortar and pestle

For disruption using a mortar and pestle, freeze the tissue sample immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen. Transfer the suspension (tissue powder and liquid nitrogen) into a liquid-nitrogen-cooled, appropriately sized tube and allow the liquid nitrogen to evaporate without allowing the sample to thaw. Add lysis buffer and continue as quickly as possible with the homogenization according to one of the two methods below.

Note: Grinding the sample using a mortar and pestle will disrupt the sample but will not homogenize it. Homogenization must be performed afterwards.

Homogenization using a syringe and needle

Cell and tissue lysates can be homogenized using a syringe and needle. Lysate is passed through a 20-gauge (0.9 mm) needle attached to a sterile plastic syringe at least 5–10 times or until a homogeneous lysate is achieved. Increasing the volume of lysis buffer may be required to facilitate handling and minimize loss.

Limitations of small samples

When purifying RNA from particularly small samples, the amounts of RNA may be too small for quantification by spectrophotometry or even fluorometric assays. In this case, quantitative real-time RT-PCR should be used for quantification. When purifying RNA from less than 100 cells, stochastic problems with respect to copy number can occur. This is because some RNA transcripts may be present at very low copy numbers per cell or only in a fraction of all cells in the sample of interest.

Protocol: Purification of Total RNA from Animal and Human Cells

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. The maximum amount depends on:

- The RNA content of the cell type
- The RNA binding capacity of the RNeasy UCP MinElute spin column (45 µg RNA)
- The volume of Buffer RULT required for efficient lysis

In addition, cellular debris can reduce the binding capacity of the RNeasy UCP MinElute spin column. If processing a cell type not listed in Table 2 and if there is no information about its RNA content, we recommend starting with no more than 5 x 10^5 cells.

Table 4. Growth area and number of HeLa cells in various culture vessels

Cell culture vessel/multiwell plates	Growth area (cm^2)*	Number of cells†
96-well	0.32–0.6	4–5 x 10^4
48-well	1	1 x 10^5
24-well	2	2.5 x 10^5
12-well	4	5 x 10^5
6-well	9.5	1 x $10^{6‡}$
Dishes, 35 mm	8	1 x $10^{6‡}$
Flasks, 40–50 ml	25	3 x $10^{6‡}$

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

† Cell numbers are given for HeLa cells (approximate length = 15 µm), assuming confluent growth. Numbers will vary for different types of animal and human cells, which vary in length from 10–30 µm.

‡ This number of cells exceeds the binding capacity of the RNeasy UCP MinElute spin columns. To process these many cells, split the lysate into appropriate aliquots (≤5 x 10^5 cells each) and load them onto separate RNeasy UCP MinElute spin columns.

Note: Do not overload the RNeasy UCP MinElute spin column, as this will significantly reduce RNA yield and purity.

Counting cells is the most accurate way to quantitate the amount of starting material. As a guide, the number of HeLa cells obtained in various culture vessels after confluent growth is given in Table 4.

Important points before starting

- If using the RNeasy UCP Micro Kit for the first time, read "Important Notes" (page 10).
- If using the TissueRuptor II, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual* and *TissueRuptor Handbook*.
- Cell pellets can be stored at –70°C for later use or used directly in the procedure.
- Determine the number of cells before freezing. Frozen cell pellets should be thawed slightly so that they can be dislodged by flicking the tube in step 2.
- Homogenized cell lysates from step 3 can be stored at –70°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. If any insoluble material is visible, centrifuge for 5 min at 3000–5000 x *g*. Transfer the supernatant to a new RNase-free glass or polypropylene tube and continue with step 4.
- Cells stored in RNAprotect Cell Reagent can also be used in the procedure. Transfer the entire sample, including any material deposited at the bottom of the storage vessel, to a centrifuge tube. Pellet the cells by centrifuging for 5 min at 5000 x *g*, and remove the supernatant by pipetting (if necessary, thaw the sample before centrifuging). Proceed immediately to step 2.
- Buffer RULT and Buffer RUWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for Safety Information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- Before using the kit for the first time, prepare aliquots of 70% and 80% ethanol for use with the RNeasy UCP Micro Kit. Use RNase-free water and 96–100% ethanol to minimize additional sources of contamination.

  70% ethanol:

  Mix 21 ml ethanol (96–100%) with 9 ml RNase-free water, resulting in 30 ml 70% ethanol, sufficient for 50 RNeasy UCP Micro preps.

  80% ethanol:

  Mix 24 ml ethanol (96–100%) with 6 ml RNase-free water, resulting in 30 ml 80% ethanol, sufficient for 50 RNeasy UCP Micro preps.

- If purifying RNA from cell lines rich in RNases, we recommend adding β-ME to Buffer RULT before use. Add 10 µl β-ME per1 ml Buffer RULT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RULT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20 µl of 2 M DTT per 1 ml Buffer RULT. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RULT containing DTT can be stored at room temperature for up to 1 month.
- Buffer RUPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RULT may form a precipitate during storage. If necessary, redissolve by warming and then place at room temperature.
- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. 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. Mix gently by inverting the vial. Do not vortex. For long-term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots and store at –30 to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. Harvest cells according to step 1a or 1b.

   1a. Cells grown in suspension (do not use more than 5 x 10^5 cells):
   Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g* in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration and proceed to step 2.

   Note: Incomplete removal of cell culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy UCP MinElute membrane. Both effects may reduce RNA yield.

   1b. Cells grown in a monolayer (do not use more than 5 x 10^5 cells):
   Cells can either be lysed directly in the cell culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell culture flasks should always be trypsinized.

   To lyse cells directly: Determine the number of cells. Completely aspirate the cell culture medium and proceed immediately to step 2.

   Note: Incomplete removal of cell culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy UCP MinElute membrane. Both effects may reduce RNA yield.

   To trypsinize and collect cells: Determine the number of cells. Aspirate the medium and wash the cells with PBS. Aspirate the PBS and add 0.1–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x *g* for 5 min. Completely aspirate the supernatant and proceed to step 2.

   Note: Incomplete removal of cell culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy UCP MinElute membrane. Both effects may reduce RNA yield.

2. Disrupt the cells by adding Buffer RULT. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add 350 µl Buffer RULT (if processing ≤1 x 10^5 cells, add 75 µl Buffer RULT instead). Vortex or pipet to mix and proceed to step 3.

   Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields.

   For direct lysis of cells grown in a monolayer, add 350 µl Buffer RULT to the cell culture dish (if processing ≤1 x 10^5 cells, especially in multiwell plates or cell culture dishes, 75 µl Buffer RULT can be added instead). Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix and ensure that no cell clumps are visible before proceeding to step 3.

3. Homogenize the lysate according to step 3a or 3b. See "Disrupting and homogenizing starting material", page 13, for more details on homogenization. If processing ≤1 x 10^5 cells, homogenize by vortexing for 1 min. After homogenization, proceed to step 4.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the RNeasy UCP MinElute spin column. Homogenization with the TissueRuptor II homogenizer generally results in higher RNA yields than with a syringe and needle.

   3a. Place the tip of the TissueRuptor Disposable Probe into the lysate and operate the TissueRuptor II at full speed until the lysate is homogenous (usually 30 s). Proceed to step 4.

   Note: To avoid damaging the TissueRuptor II and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer.

   3b. Pass the lysate at least 5 times through a blunt 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Proceed to step 4.

4. Add 1 volume of 70% ethanol to the lysate, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 5.

   Note: The volume of lysate may be less than 350 µl due to loss during homogenization. If only 75 µl of Buffer RULT was used in step 2, then add only 75 µl of 70% ethanol in this step.

   Note: When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.

5. Transfer the sample, including any precipitate that may have formed, to an RNeasy UCP MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.*

6. Add 350 µl Buffer RUWT to the RNeasy UCP MinElute spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin-column membrane. Discard the flow-through.*

   Reuse the collection tube in step 9.

7. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube.

   Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.

8. Add the DNase I incubation mix (80 µl) directly to the RNeasy MinElute spin-column membrane and place on the benchtop (20–30°C) for 15 min.

   Note: Be sure to add the DNase I incubation mix directly to the RNeasy UCP MinElute spin-column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

* Flow-through contains buffers RULT or RUWT and is therefore not compatible with bleach. See page 5 for Safety Information.

9. Add 350 µl Buffer RUWT to the RNeasy UCP MinElute spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin-column membrane. Discard the flow-through* and collection tube.

10. Place the RNeasy UCP MinElute spin column in a new 2 ml collection tube (supplied).

11. Add 500 µl Buffer RUPE to the spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin-column membrane. Discard the flow-through.*

    Reuse the collection tube in step 12.

    Note: Buffer RUPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RUPE before use (see "Things to do before starting", page 17).

12. Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin-column membrane. Discard the flow-through and collection tube.

    Note: After centrifugation, carefully remove the RNeasy UCP MinElute spin column from the collection tube so that the column does not contact the flow-through; otherwise, carryover of ethanol will occur.

* Flow-through contains buffers RULT or RUWT and is therefore not compatible with bleach. See page 5 for Safety Information.

13. Place the RNeasy UCP MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column and centrifuge at full speed for 5 min. Discard the flow-through and collection tube.

    To avoid damaging the lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

    It is important to dry the spin-column membrane because residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

14. Place the RNeasy UCP MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl ultra-clean water directly to the center of the spin-column membrane. Close the lid and centrifuge for 1 min at full speed to elute the RNA.

    As little as 10 µl ultra-clean water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%.

    Do not elute with less than 10 µl ultra-clean water, as the spin-column membrane will not be sufficiently hydrated.

    The dead volume of the RNeasy UCP MinElute spin column is 2 µl: elution with 14 µl ultra-clean water results in a 12 µl eluate.

Protocol: Purification of Total RNA from Animal and Human Tissues

This protocol is for the purification of RNA from most animal and human tissues. This protocol is not suited for fibrous or fatty tissues.

Determining the correct amount of starting material

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. A maximum amount of 5 mg fresh or frozen tissue or 2–3 mg RNAprotect Tissue Reagent- or Allprotect-stabilized tissue (which is partially dehydrated) can generally be processed. For most tissues, the RNA binding capacity of the RNeasy UCP MinElute spin column and the lysing capacity of Buffer RULT will not be exceeded by these amounts.

Typical RNA yields from various tissues are given in Table 2 (page 11). Some tissues, such as spleen, parts of the brain, lung, and thymus, tend to form precipitates during the procedure; however, this does not affect RNA purification.

Note: Do not overload the RNeasy UCP MinElute spin column, as this will significantly reduce RNA yield and quality.

Weighing tissue is the most accurate way to quantitate the amount of starting material. As a guide, a 1.5 mm cube (3.4 mm^3) of most animal tissues weighs 3.5–4.5 mg.

Important points before starting

- If using the RNeasy Micro Kit for the first time, read "Important Notes" (page 10).
- If using the TissueRuptor II, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual* and *TissueRuptor Handbook*.
- If using the TissueLyser II, ensure that you are familiar with operating it by referring to the operating instructions and the *TissueLyser Handbook*.
- For optimal results, stabilize harvested tissues immediately in RNAprotect Tissue Reagent (see the *RNAprotect Tissue Handbook*) or Allprotect Tissue Reagent (see the *Allprotect Tissue Reagent Handbook*). Tissues can be stored in these reagents at 37°C for up to 1 day, at 15–25°C for up to 7 days or at 2–8°C for up to 4 weeks (RNAprotect) or 6 months (Allprotect). Alternatively, tissues can be stored at –30°C to –15°C or –80°C.
- Fresh, frozen or RNAprotect Tissue Reagent- or Allprotect-stabilized tissues can be used. Tissues can be stored at –70°C for several months. Flash-freeze tissues in liquid nitrogen and immediately transfer to –70°C. Do not allow tissues to thaw during weighing or handling prior to disruption in Buffer RULT. Homogenized tissue lysates from step 3 can also be stored at –70°C for several months. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 4. Avoid prolonged incubation, which may compromise RNA integrity.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- Before using the kit for the first time, prepare aliquots of 70% and 80% ethanol for use with the RNeasy UCP Micro Kit. Use RNase-free water and 96–100% ethanol to minimize additional sources of contamination.

  70% ethanol:

  Mix 21 ml ethanol (96–100%) with 9 ml RNase-free water, resulting in 30 ml 70% ethanol, sufficient for 50 RNeasy UCP Micro preps.

  80% ethanol:

  Mix 24 ml ethanol (96–100%) with 6 ml RNase-free water, resulting in 30 ml 80% ethanol, sufficient for 50 RNeasy UCP Micro preps.

- β-ME must be added to Buffer RULT before use. Add 10 µl β-ME per 1 ml Buffer RULT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RULT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20 µl of 2 M DTT per 1 ml Buffer RULT. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RULT containing DTT can be stored at room temperature for up to 1 month.
- Buffer RUPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RULT may form a precipitate during storage. If necessary, redissolve by warming and then place at room temperature.
- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. 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. Mix gently by inverting the vial. Do not vortex.

- For long-term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots and store at –30 to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue. Do not use more than 5 mg. Proceed immediately to step 2.

   Weighing tissue is the most accurate way to determine the amount. If necessary, cut the tissue on a clean surface and weigh the piece to be used.

   For RNAprotect Tissue Reagent- or Allprotect-stabilized tissues: Remove the tissue from the stabilization reagent using forceps and be sure to remove any crystals that may have formed. RNA in RNAprotect Tissue Reagent- or Allprotect-stabilized tissues is protected during cutting and weighing of tissues at room temperature (15–25°C). It is not necessary to cut the tissues on ice, dry ice or in a refrigerated room. Remaining tissues can be stored in RNAprotect Tissue Reagent or Allprotect Reagent. Previously stabilized tissues can be stored at –80°C without reagent.

   For unstabilized fresh or frozen tissues: RNA in harvested tissues is not protected until the tissues are treated with RNAprotect Tissue Reagent or Allprotect Reagent, flash-frozen or disrupted and homogenized in step 2. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible. Remaining fresh tissues can be placed into RNAprotect Tissue Reagent to stabilize RNA or in Allprotect Tissue Reagent to stabilize DNA, RNA, and protein. Remaining frozen tissue can be refrozen and stored at –80°C, because RNAprotect Tissue Reagent diffuses slowly through thawing tissue, preventing RNA degradation.

2. Disrupt the tissue and homogenize the lysate in Buffer RULT (do not use more than 5 mg tissue) according to step 2a or 2b.

   See “Disrupting and homogenizing starting material”, page 13, for more details on disruption and homogenization.

   Note: Ensure that β-ME (or DTT) is added to Buffer RULT before use (see “Things to do before starting”, page 26).

   After storage in RNAprotect Tissue Reagent or Allprotect Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization using standard methods is usually sufficient.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the RNeasy UCP MinElute spin column. Homogenization with the TissueRuptor II or TissueLyser II generally results in higher RNA yields compared to other methods.

   2a. Disruption and homogenization using the TissueRuptor II: Place the tissue in a suitably sized vessel. Add 350 µl Buffer RULT.

   Note: Use a suitably sized vessel with sufficient extra headspace to accommodate foaming, which may occur during homogenization. Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.

   Place the tip of the disposable probe into the vessel and operate the TissueRuptor at full speed until the lysate is homogeneous (usually 30 s).

   Proceed to step 3.

   Note: To avoid damaging the TissueRuptor II and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer. Foaming may occur during homogenization. If this happens, let the homogenate stand at room temperature for 2–3 min until the foam subsides before continuing with the procedure.

2b. Disruption and homogenization using the TissueLyser II: Place the tissues in 2 ml microcentrifuge tubes containing one stainless steel bead (5 mm mean diameter). If handling fresh or frozen tissue samples, keep the tubes on dry ice. Place the tubes at room temperature. Immediately add 350 µl Buffer RULT per tube. Place the tubes in the TissueLyser Adapter Set 2 x 24. Operate the TissueLyser II for 2 min at 20 Hz. The time depends on the tissue being processed and can be extended until the tissue is completely homogenized. Rearrange the collection tubes so that the outermost tubes are innermost and the innermost tubes are outermost. Operate the TissueLyser II for another 2 min at 20 Hz. Rearranging the tubes allows even homogenization.

Proceed to step 3.

Do not reuse the stainless-steel beads.

3. Centrifuge the lysate for 3 min at full speed. Carefully transfer the supernatant to a new microcentrifuge tube (not supplied) by pipetting. Use only this supernatant (lysate) in subsequent steps.

   In some preparations, very small amounts of insoluble material will be present after the 3 min centrifugation, making the pellet invisible.

4. Add 1 volume (usually 350 µl) of 70% ethanol to the lysate and mix well by pipetting. Do not centrifuge. Proceed immediately to step 5.

   Note: The volume of 70% ethanol to add may be less than 350 µl if some lysate was lost during homogenization.

   Note: Precipitates may be visible after addition of ethanol, but this does not affect the procedure.

5. Transfer the sample, including any precipitate that may have formed, to an RNeasy UCP MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.* Reuse the collection tube in step 6.

* Flow-through contains buffers RULT or RUWT and is therefore not compatible with bleach. See page 5 for Safety Information.

6. Add 350 µl Buffer RUWT to the RNeasy MinElute spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin-column membrane. Discard the flow-through.*

   Reuse the collection tube in step 9.

7. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube.

   Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.

8. Add the DNase I incubation mix (80 µl) directly to the RNeasy UCP MinElute spin-column membrane and place on the benchtop (20–30°C) for 15 min.

   Note: Be sure to add the DNase I incubation mix directly to the RNeasy UCP MinElute spin-column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

9. Add 350 µl Buffer RUWT to the RNeasy UCP MinElute spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin-column membrane. Discard the flow-through* and collection tube.

10. Place the RNeasy UCP MinElute spin column in a new 2 ml collection tube (supplied). Add 500 µl Buffer RUPE to the spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin-column membrane. Discard the flow-through.

    Reuse the collection tube in step 11.

    Note: Buffer RUPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RUPE before use (see "Things to do before starting", page 26).

* Flow-through contains buffers RULT or RUWT and is therefore not compatible with bleach. See page 5 for Safety Information.

11. Add 500 µl of 80% ethanol to the RNeasy UCP MinElute spin column. Close the lid and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin-column membrane. Discard the flow-through and collection tube.

    Note: After centrifugation, carefully remove the RNeasy UCP MinElute spin column from the collection tube so that the column does not contact the flow-through; otherwise, carryover of ethanol will occur.

    Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column and centrifuge at full speed for 5 min. Discard the flow-through and collection tube.

    To avoid damaging the lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

    It is important to dry the spin-column membrane because residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

12. Place the RNeasy UCP MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl ultra-clean water directly to the center of the spin-column membrane. Close the lid and centrifuge for 1 min at full speed to elute the RNA.

    As little as 10 µl ultra-clean water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 µl ultra-clean water, as the spin-column membrane will not be sufficiently hydrated.

    The dead volume of the RNeasy UCP MinElute spin column is 2 µl: elution with 14 µl ultra-clean water results in a 12 µl eluate.

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 in our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		Comments and suggestions
Clogged RNeasy MinElute spin column		
a)	Inefficient disruption	See "Disrupting and homogenizing starting material" (page 13) for details on disruption and homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see the individual protocols) and/or increase the homogenization time.
b)	Too much starting material	Reduce the amount of starting material (see the individual protocols). It is essential to use the correct amount of starting material.
c)	Centrifugation temperature too low	The centrifugation temperature should be 20–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the spin column. If this happens, set the centrifugation temperature to 25°C. Warm the lysate to 37°C before transferring it to the RNeasy MinElute spin column.
Low RNA yield		
a)	Insufficient disruption and homogenization	See "Disrupting and homogenizing starting material" (page 13) for details on disruption and homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see the individual protocols) and/or increase the volume of lysis buffer and the homogenization time.
b)	Too much starting material	In subsequent preparations, reduce the amount of starting material (see the individual protocols). It is essential to use the correct amount of starting material.
c)	RNA still bound to spin column	Repeat RNA elution, but incubate the membrane of the RNeasy MinElute spin column on the benchtop for 10 min with RNase-free water before centrifuging.

	Comments and suggestions
d) Ethanol carryover	After the wash with 80% ethanol, be sure to centrifuge at full speed for 5 min to dry the RNeasy MinElute spin-column membrane. After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through; otherwise, carryover of ethanol will occur.
e) 80% ethanol not made with RNase-free water	The 80% ethanol used to wash the RNeasy MinElute spin-column membrane must be free of RNases. Be sure to prepare the 80% ethanol using ethanol (96–100%) and the RNase-free water, as described in "Things to do before starting" in each protocol.
f) RNase-free water incorrectly dispensed	Pipet RNase-free water to the center of the RNeasy MinElute spin-column membrane to ensure that the membrane is completely covered.
Low A_{260}/A_{280} value	
Water used to dilute RNA A_{260}/A_{280} measurement	Use 10 mM Tris·Cl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity.
RNA degraded	
a) Inappropriate handling of starting material	Ensure that tissue samples are properly stabilized and stored in RNAprotect Tissue Reagent or Allprotect Tissue Reagent. For frozen cell pellets or frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the RNeasy procedure quickly, especially the first few steps.
b) RNase contamination	Although all RNeasy buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the RNeasy procedure or later handling. Do not put RNA samples into a vacuum dryer or microcentrifuge that has been used in DNA preparations where RNases may have been used.
c) 80% ethanol not made with RNase-free water	The 80% ethanol used to wash the RNeasy MinElute spin-column membrane must be free of RNases. Be sure to prepare the 80% ethanol using ethanol (96–100%) and the RNase-free water, as described in "Things to do before starting" in each protocol.
DNA contamination in downstream experiments	
No DNase treatment	Be sure to perform the on-column DNase digestion as described in the protocols.

		Comments and suggestions
RNA does not perform well in downstream experiments		
a)	Ethanol carryover	After the wash with 80% ethanol, be sure to centrifuge at full speed for 5 min to dry the RNeasy MinElute spin-column membrane. After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through; otherwise, carryover of ethanol will occur.
b)	Salt carryover during elution	Ensure that buffers are at 20–30°. Ensure that the correct buffer is used for each step of the procedure. When reusing collection tubes between washing steps, remove residual flow-through from the rim by blotting on clean paper towels.

Ordering Information

Product	Contents	Cat. no.
RNeasy UCP Micro Kit (50)	50 RNeasy UCP MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free DNase I, RNase-Free Reagents and UCP Buffers RULT, RUWT, RUPE.	73934
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	9002864
Starter Pack, QIAcube	200 µl filter-tips (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), 1.5 ml elution tubes (240), rotor adapter holder (1)	990395
Related products		
QIAamp UCP DNA Micro Kit (50)	For 50 preps: QIAamp UCP MinElute spin columns, QIAGEN Proteinase K, Buffers	56204
RNAprotect Tissue Reagent (50 ml)	50 ml RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104
RNAprotect Tissue Reagent (250 ml)	250 ml RNAprotect Tissue Reagent for stabilization of RNA in 125 x 200 mg tissue samples	76106
RNAprotect Tissue Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 50 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNAprotect Tissue Reagent each	76154

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training, and preventive subscription. Contact your local sales representative to learn about your options.

Product	Contents	Cat. no.
RNAprotect Tissue Tubes (20 x 5 ml)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNAprotect Tissue Reagent each	76163
TissueRuptor II	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes.	9002757
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor II.	990890
TissueLyser II	Universal laboratory mixer-mill disruptor.	85300
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II.	69982
Stainless Steel Beads 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system.	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter).	69965

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Document Revision History

Date	Changes
12/2016	Initial revision
01/2020	Updated text, ordering information and intended use for QIAcube Connect.
03/2021	Updated branding of RNA protection products and the Ordering Information section.

Notes

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