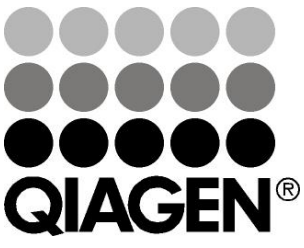
June 2015

# miRNeasy® 96 Handbook

For high-throughput purification of total RNA, including miRNA, from animal cells and tissues




Sample & Assay Technologies

# QIAGEN Sample and Assay Technologies

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**QIAGEN sets standards in**:

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- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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# Contents

# Kit Contents

| **miRNeasy 96 Kit** | **(4)** |
|---|---|
| **Catalog no.** | **217061** |
| **Number of preps** | **4 x 96** |
| RNeasy® 96 Plates | 4 |
| Register Cards (96-well) | 4 |
| Collection Microtubes (racked) | 4 x 96 |
| Collection Microtube Caps | 100 x 8 |
| S-Blocks | 6 |
| Elution Microtubes CL | 4 x 96 |
| Elution Microtube Caps (strips) | 50 x 8 |
| AirPore Tape Sheets | 3 x 5 |
| Buffer RWT*† | 2 x 80 ml |
| Buffer RPE‡ | 4 x 65 ml |
| RNase-Free Water | 2 x 50 ml |
| QIAzol® Lysis Reagent*§ | 2 x 200 ml |
| Quick-Start Protocol | 1 |

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

† Buffer RWT is supplied as a concentrate. Before using for the first time, add 2 volumes of ethanol (96%–100%) as indicated on the bottle to obtain a working solution.

‡ Buffer RPE 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.

§ Packaged separately.

Additional QIAzol Lysis Reagent, S-Blocks, Elution Microtubes CL, Collection Microtubes (racked), and AirPore Tape Sheets are available separately. See ordering information (page 58).

# Storage

The miRNeasy 96 Kit should be stored dry at room temperature (15–25°C). All components are stable for at least 9 months under these conditions.

QIAzol Lysis Reagent can be stored at room temperature or at 2–8°C.

## Intended Use

The miRNeasy 96 Kit is intended for molecular biology applications. 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 format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

## Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of the miRNeasy 96 Kit is tested against predetermined specifications to ensure consistent product quality.

# Introduction

To date, the majority of gene expression studies have focused on mRNA levels. However in recent years, interest in smaller RNA species, such as miRNA, has increased. Most commercial RNA purification kits do not recover RNA molecules smaller than ~200 nucleotides. The miRNeasy 96 Kit is designed for purification of total RNA, including miRNA and other small RNA molecules, from cultured cells and various animal tissues. The miRNeasy 96 Kit enables efficient RNA preparation from 96 or 192 samples in parallel.

In less than 2 hours (including homogenization), 96 high-purity RNA samples can be obtained. The miRNeasy 96 procedure replaces current time-consuming and tedious methods involving alcohol-precipitation steps or large numbers of washing steps. The purified RNA is ready to use in any downstream application, including:

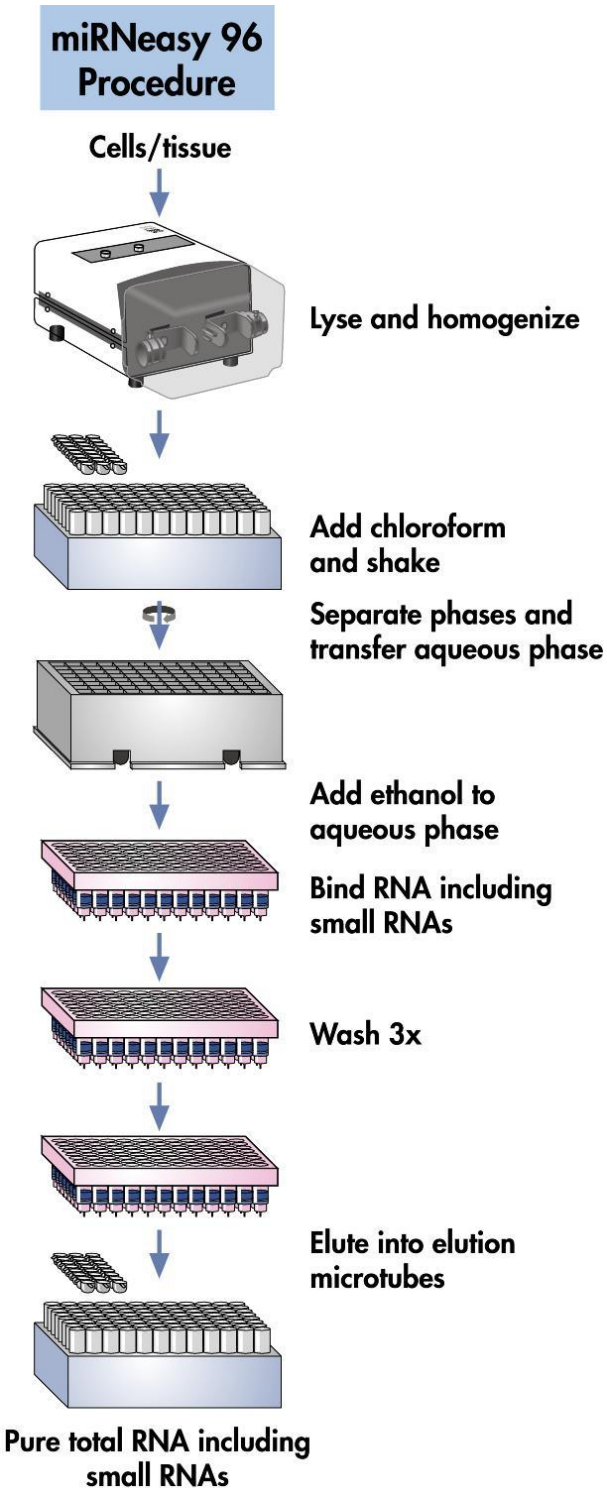
- RT-PCR
- Quantitative RT-PCR
- Differential display
- cDNA synthesis
- Northern, dot, and slot blot analyses
- Primer extension
- Poly $A^+$ RNA selection
- RNase/S1 nuclease protection
- Microarrays

## Principle and procedure

The miRNeasy 96 Kit combines phenol/guanidine-based sample lysis and silica-membrane–based RNA purification with the speed of vacuum and/or spin processing (see flowchart page 8). QIAzol Lysis Reagent, included in the kit, is a monophasic solution of phenol and guanidine thiocyanate, designed to facilitate lysis of tissues, to inhibit RNases, and also to remove most of the cellular DNA and proteins from the lysate by organic extraction.

Cultured animal cells are lysed by vortexing in QIAzol Lysis Reagent. Animal tissue is lysed using QIAzol Lysis Reagent and the TissueLyser II, which provides rapid and parallel disruption of multiple samples. After phase separation by centrifugation and recovery of the aqueous phase, ethanol is added to provide appropriate binding conditions for recovery of all RNA, including miRNA and other small RNA molecules ≥18 nucleotides. Samples are then applied to the wells of the RNeasy 96 plate, where total RNA binds and contaminants are efficiently washed away. High-quality RNA is then eluted in a small volume of water, ready for use in any downstream application.

For enrichment of miRNAs and other small RNAs (less than ~200 nt) in a separate fraction, a specialized protocol is provided in Appendix A, page 45. Enrichment of small RNAs in a separate fraction may be advantageous for certain applications where mRNA and rRNA could lead to increased background. For this specialized protocol, 2 RNeasy 96 plates are required for every 96 samples.


miRNeasy 96 Procedure
Cells/tissue
Lyse and homogenize
Add chloroform and shake
Separate phases and transfer aqueous phase
Add ethanol to aqueous phase
Bind RNA including small RNAs
Wash 3x
Elute into elution microtubes
Pure total RNA including small RNAs

## Description of protocols

The protocols in this handbook provide 2 different handling options, using either a combination of vacuum and spin technology or spin technology alone. Both handling options provide high yields of high-quality RNA.

### I. Vacuum/spin technology

Using vacuum/spin technology, all protocol steps from the binding step until the final wash step are performed on the QIAvac 96 vacuum manifold (see page 13). Phase separation, membrane drying, and the elution steps are performed in the Centrifuge 4–16K (see page 11). The Plate Rotor 2 x 96 holds 2 RNeasy 96 plates, allowing up to 192 RNA samples to be prepared in parallel. Residual traces of salt are removed by centrifugation in the final wash step. RNA purified using vacuum/spin technology can be used for any nonenzymatic or enzymatic downstream application, including quantitative RT-PCR analysis with QuantiTect® technology.

### II. Spin technology

Using spin technology, all protocol steps are performed in the Centrifuge 4–16K (see page 11). The Plate Rotor 2 x 96 holds 2 RNeasy 96 plates, allowing up to 192 RNA samples to be prepared in parallel. RNA purified using spin technology can be used for any nonenzymatic or enzymatic downstream application, including quantitative RT-PCR analysis with QuantiTect technology.

# 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

- Multichannel pipet with tips. For the most efficient sample processing in the miRNeasy 96 protocol, we recommend the use of an electric multichannel pipet with a minimum capacity of 650 µl per pipet tip. Matrix® Impact® cordless electronic multichannel pipets have a unique expandable tip-spacing system allowing the user to transfer liquid directly from racks of tubes to 96-well microplates. Matrix Impact pipets can be purchased from Matrix Technologies Corporation (www.matrixtechcorp.com).*
- Reagent reservoirs for multichannel pipets (**Note**: Make sure that reagent reservoirs used for chloroform are chloroform-resistant.)
- Disposable gloves
- Centrifuge 4–16K (see page 11)
- Plate Rotor 2 x 96 (see page 11)
- QIAGEN's TissueLyser II (see page 11) or a rotor–stator homogenizer
- Chloroform (without added isoamyl alcohol)
- Ethanol (70% and 96–100%)†
- Dry ice
- For animal tissues: RNA*later*® RNA Stabilization Reagent (see ordering information, page 58) or liquid nitrogen
- **Optional:** additional S-Blocks (cat. no. 19585). Two S-Blocks are supplied with the kit for use as waste-trays. If several RNeasy 96 plates are processed per day, it may be convenient to keep extra S-Blocks on hand.

### For protocol using vacuum/spin technology

- QIAvac 96 vacuum manifold (see Figure 1, page 12)
- Vacuum source capable of generating a vacuum pressure of –800 to –900 mbar (see pages 13–16). Vacuum Pumps (capacity 34 liter/min) can be purchased from QIAGEN (see ordering information, page 58)

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

† Do not use denatured alcohol, which contains other substances such as methanol and methylethylketone.

### Optional reagents

- RNase-Free DNase Set (cat. no. 79254), containing RNase-free DNase I, Buffer RDD, and RNase-free water. For optional on-plate DNase digestion, the miRNeasy 96 procedure requires 2 RNase-Free DNase Sets per 96-well plate.
- RNase inhibitors (available from Promega, Applied Biosystems, Amersham Pharmacia, and Stratagene)*

## TissueLyser II

The TissueLyser II provides high-throughput processing for simultaneous, rapid, and effective disruption of up to 192 biological samples, including all types of animal tissue. Processing of up to 2 x 96 samples takes as little as 2–5 minutes.

Disruption and homogenization using the TissueLyser II gives yields comparable or better than with traditional rotor–stator homogenization methods. With rotor–stator homogenization, the samples must be processed individually, and the rotor–stator homogenizer must be cleaned after each sample to prevent cross-contamination. In contrast, the TissueLyser II provides simultaneous disruption for high-throughput processing of a variety of animal tissues.

The TissueLyser II includes a number of different accessories for ease of use with different sample sizes and throughputs. In the miRNeasy 96 procedure, the TissueLyser Adapter Set 2 x 96 allows simultaneous processing of up to 192 samples in collection microtubes. Stainless steel beads with a diameter of 5 mm are optimal to use for animal tissues in combination with the miRNeasy 96 Kit. The TissueLyser 5 mm Bead Dispenser, 96-Well, is also available to conveniently deliver 96 beads in parallel into collection microtubes. See page 58 for ordering information.

## Centrifuge 4–16K

All miRNeasy 96 protocols utilize a streamlined centrifugation procedure that allows preparation of RNA from up to 2 x 96 samples in parallel for direct use in any downstream application. For optimal handling, QIAGEN, in cooperation with the centrifuge manufacturer Sigma Laborzentrifugen GmbH, has developed a centrifugation system consisting of the Plate Rotor 2 x 96 and the refrigerated table-top Centrifuge 4–16K (see ordering information, page 58). A temperature of 4°C is necessary during phase separation for optimal removal of genomic DNA. A wide range of other rotors can be used with Centrifuge 4–16K in addition to the Plate Rotor 2 x 96.

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Standard table-top centrifuges and 96-well–microplate rotors are not suitable for the miRNeasy 96 procedure. Usually 96-well–microplate buckets are not deep enough to carry the complete RNeasy assembly and may not swing out properly during centrifugation. Furthermore, high *g*-forces (>5500 x *g*) are required for optimal performance of the miRNeasy 96 procedure.

For further information about the centrifuge and rotor, please contact QIAGEN or your local distributor.

**Warning**: Do not centrifuge the Plate Rotor 2 x 96 metal holders without the RNeasy 96 plates and S-Blocks or elution microtubes. If unsupported, the holders will collapse under high *g*-force. Therefore, remove the holders during test runs. Standard 96-well microplates may be centrifuged in the holders if a *g*-force of 500 x *g* is not exceeded.

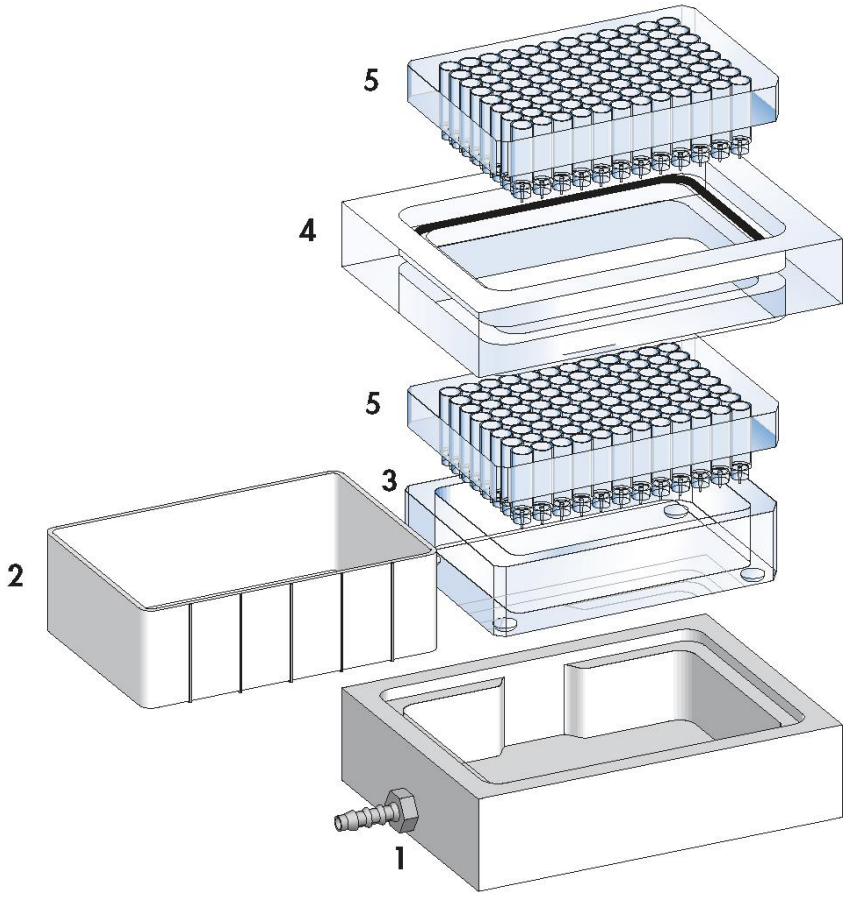



**Figure 1. QIAvac 96 vacuum manifold.** Components of the QIAvac 96 vacuum manifold

1. QIAvac base, which holds a waste tray, a plate holder, or a microtube rack
2. Waste tray
3. Plate holder (shown with 96-well plate) — not used in miRNeasy 96 protocols
4. QIAvac 96 top plate with aperture for 96-well plate
5. 96-well plate — not included with QIAvac 96. Included in the miRNeasy 96 Kit.

## QIAvac 96 handling guidelines

QIAvac 96 facilitates the miRNeasy 96 procedure by providing a convenient, modular vacuum manifold (see Figure 1, page 12) for use with the miRNeasy 96 Kit. The following recommendations should be followed when handling the QIAvac 96 vacuum manifold.

- QIAvac 96 operates with house vacuum or a vacuum pump. If the house vacuum is weak or inconsistent, we recommend using a vacuum pump with a capacity of 18 liter/min. Use of insufficient vacuum pressure may reduce RNA yield and purity.
- A vacuum pressure of –800 to –900 mbar should develop when an RNeasy 96 plate sealed with tape is used on the QIAvac 96. Vacuum pressures exceeding –900 mbar should be avoided. The vacuum pressure is the pressure difference between the inside of the manifold and the atmosphere (standard atmospheric pressure: 1013 millibar or 760 mm Hg) and can be regulated and measured using a pressure gauge or vacuum regulator (see ordering information, page 58). Vacuum recommendations are given in negative units to indicate the required reduction in pressure with respect to the atmosphere. Table 1 (page 14) provides pressure conversions to other units.
- Between loading steps, the vacuum must be switched off and the manifold ventilated to maintain uniform conditions for each sample. This can be done with a vacuum regulator (see ordering information, page 58) inserted between the vacuum source and the QIAvac 96 vacuum manifold.
- Wear safety glasses when working near a manifold under pressure.
- For safety reasons, do not use 96-well plates that have been damaged in any way.
- Always place the QIAvac 96 vacuum manifold on a secure bench top or work area. If dropped, the manifold may crack.
- Always store the QIAvac 96 vacuum manifold clean and dry. To clean, simply rinse all components with water and dry with paper towels. Do not air dry, as the screws may rust and need to be replaced. Do not use abrasives. Finally, wipe manifold components with paper towels wetted with 70% ethanol, and dry with fresh paper towels.
- The QIAvac 96 vacuum manifold and components are not resistant to ethanol, methanol, or other organic solvents when exposed for long periods. If solvents are spilled on the unit, rinse thoroughly with distilled water after the RNeasy preparation. Ensure that no residual buffers remain in the vacuum manifold.

- To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 96 vacuum manifold. The spring lock on the top plate and the self-sealing gasket provide an airtight seal when vacuum is applied to the assembled unit. To maximize gasket life, rinse the gasket free of salts and buffers after each use and dry with paper towels before storage.

**Table 1. Pressure conversions**

| To convert from millibars (mbar) to: | Multiply by: |
|---|---|
| Millimeters of mercury (mm Hg) | 0.75 |
| Kilopascals (kPa) | 0.1 |
| Inches of mercury (inch Hg) | 0.0295 |
| Torrs (Torr) | 0.75 |
| Atmospheres (atmos) | 0.000987 |
| Pounds per square inch (psi) | 0.0145 |

# Important Notes

## Determining the amount of starting material

Using the correct amount of starting material is essential in order to obtain high yields of pure RNA with the miRNeasy 96 Kit. The maximum amount that can be used is limited by:

- The volume of QIAzol Lysis Reagent required for efficient lysis
- The RNA binding capacity of the RNeasy 96 plate wells (100 µg)
- The type of sample and its RNA content

The miRNeasy 96 procedure is optimized for use with a maximum of 50 mg animal tissue (flash frozen). With adipose tissue, up to 100 mg can be used. With liver, thymus, spleen, or RNA*later* stabilized tissues,* only 25 mg should be used in order to avoid clogging the RNeasy 96 plate.

Table 2 gives specifications for the RNeasy 96 plate. Each well of the RNeasy 96 plate has a maximum binding capacity of 100 µg of RNA, but actual RNA yields depend on the sample type used. Table 4 (page 17) gives examples of expected RNA yields from various sample types.

**Table 2. RNeasy 96 Plate specifications**

| | |
|---|---|
| Preps per plate | 96 |
| Amount of starting material | Up to 50 mg tissue (up to 100 mg for adipose tissue)<br>Up to 1 x $10^7$ cells |
| Binding capacity per well | 100 µg RNA† |
| Maximum loading volume per well | 1 ml |
| Minimum elution volume | 45 µl |

† Yields are limited by sample type and amount. The maximum binding capacity of 100 µg RNA is usually not reached.

**Note:** If the binding capacity of the RNeasy 96 plate is exceeded, yields of total RNA will not be consistent and may be less than expected. If lysis of the starting material is incomplete, yields of total RNA will be lower than expected, even if the binding capacity of the RNeasy 96 plate is not exceeded.

* See the *RNA*later *Handbook* for more information about RNA*later* RNA Stabilization Reagent.

### Determining the correct amount of starting material — cells

Use of QIAzol Lysis Reagent in the miRNeasy 96 procedure removes most of the genomic DNA and cellular protein. For this reason, up to 3 x $10^6$ cells can be processed by vortexing without further homogenization of the cell lysates.

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 3.

**Table 3. Growth area and number of HeLa cells in various culture vessels**

| Cell-culture vessel | Growth area ($cm^2$)* | Number of cells† |
|---|---|---|
| **Multiwell-plates** | | |
| ■ 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 $10^6$ |
| ■ 60 mm | 21 | 2.5 x $10^6$ |
| ■ 100 mm | 56 | 7 x $10^6$ |
| ■ 145–150 mm | 145 | 2 x $10^7$ |
| **Flasks** | | |
| ■ 40–50 ml | 25 | 3 x $10^6$ |
| ■ 250–300 ml | 75 | 1 x $10^7$ |
| ■ 650–750 ml | 162–175 | 2 x $10^7$ |

* 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. Cell numbers will vary for different kinds of animal cells, which vary in length from 10 to 30 µm.

### Determining the correct amount of starting material — tissue

Generally, this protocol can be used with a maximum of

- 50 mg flash-frozen tissue
- 25 mg liver, thymus, spleen, or RNA*later* stabilized tissue
- 100 mg adipose tissue

Average RNA yields from various sources are given in Table 4 (next page).

**Table 4. Average yields of total RNA with the miRNeasy 96 Kit**

| Sample | Average RNA yield* (µg) |
|---|---|
| **Cell culture (1 x $10^6$ cells)** | |
| NIH/3T3 | 10 |
| HeLa | 15 |
| COS-7 | 35 |
| LMH | 12 |
| Huh | 15 |
| **Mouse/rat tissue (10 mg)** | |
| Kidney | 5–40 |
| Liver | 15–80 |
| Lung | 5–15 |
| Heart | 5–25 |
| Muscle | 5–35 |
| Brain | 5–20 |
| Adipose tissue | 0.5–2.5 |
| Spleen | 15–100 |
| Intestine | 10–60 |
| Skin | 2–5 |

* Amounts can vary due to species, age, gender, physiological state, growth conditions, etc. Weighing tissue is the most accurate way to quantify the amount of starting material. However, the following may be used as a guide. A 3 mm cube (volume, 27 $mm^3$) of most animal tissues weighs 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 RNA*later* RNA Stabilization 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 will occur. It is therefore important that samples are immediately frozen in liquid nitrogen and stored at –70°C (animal tissues only), processed as soon as harvested, or immediately immersed in

RNAprotect Cell Reagent or RNA*later* RNA Stabilization Reagent. Animal cells can be pelleted and then stored at –70°C until required for RNA purification.

The procedures for harvesting and RNA protection should be carried out as quickly as possible. Frozen samples should not be allowed to thaw during handling or weighing. After disruption and homogenization in QIAzol Lysis Reagent, samples can be stored at –70°C for 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 are 2 distinct steps:

- **Disruption:** Complete disruption of plasma membranes of cells 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 significantly reduces RNA yields.
- **Homogenization:** Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Homogenization shears high-molecular–weight genomic DNA and other high-molecular–weight cellular components to create a homogeneous lysate. In the miRNeasy procedure, genomic DNA is removed by organic extraction, which makes it possible to homogenize up to 3 x$10^6$ cells by vortexing without additional homogenization. Incomplete homogenization results in inefficient binding of RNA to the RNeasy membrane, significantly reducing RNA yields.

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step. Table 5 (next page) gives an overview of different disruption and homogenization methods and is followed by a detailed description of each method. This information can be used as a guide to choose the appropriate methods for your starting material.

**Table 5. Disruption and homogenization methods**

| Sample | Disruption method | Homogenization method | Comments |
|---|---|---|---|
| Animal cells | Addition of lysis buffer | Vortexing (for ≤3 x $10^6$ cells), TissueLyser II, or rotor–stator homogenizer | If processing ≤3 x $10^6$ cells, lysate can be homogenized by vortexing. |
| Animal tissues | TissueLyser II | TissueLyser II | Simultaneously disrupts and homogenizes up to 192 samples in parallel. The TissueLyser II gives results comparable to using a rotor–stator homogenizer. |
| | Rotor–stator homogenization | Rotor–stator homogenization | Simultaneously disrupts and homogenizes individual samples. |

### 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. Disruption efficiency is influenced by:

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

Stainless steel beads with a 5 mm diameter are optimal for use with animal tissues in combination with the miRNeasy 96 Kit. All other disruption parameters should be determined empirically for each application. The protocols in this handbook give guidelines for disruption and homogenization of tissues using the TissueLyser II and stainless steel beads. For other bead mills, please refer to suppliers' guidelines for further details.

### Disruption and homogenization using rotor–stator homogenizers

Rotor–stator homogenizers thoroughly disrupt and simultaneously homogenize animal tissues in the presence of lysis buffer in 15–90 seconds depending on the toughness and size of the sample. Rotor–stator homogenizers can also be used to homogenize cell lysates.

The rotor turns at a very high speed, causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. Foaming of the sample should be kept to a minimum by using properly sized vessels, keeping the tip of the homogenizer submerged, and holding the immersed tip to the side of the tube. Rotor–stator homogenizers are available in different sizes and operate with differently sized probes.

## S-Blocks

S-Blocks are supplied with the kit. If several RNeasy 96 plates are processed per day, it may be convenient to keep extra S-Blocks on hand. See ordering information on page 58.

Fresh S-Blocks must be used to take up the aqueous phase. S-Blocks may be cleaned and reused as waste trays. **Do not reuse cleaned S-Blocks to collect the aqueous phase.**

To reuse the S-Blocks as waste trays, rinse them thoroughly with tap water and incubate for 2 hours or overnight in 0.1 N NaOH, 1 mM EDTA.* Rinse in distilled water and dry at 50°C.

**Note:** Do not use bleach. Bleach may react with residual amounts of QIAzol Lysis Reagent and Buffer RWT on the S-Blocks.

* 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.

# Protocol: Purification of Total RNA, Including Small RNAs, from Animal Cells using Vacuum/Spin Technology

### Important points before starting

- If using the miRNeasy 96 Kit for the first time, read "Important Notes" (page 15).
- It is important not to overload the RNeasy plate, as overloading will significantly reduce RNA yield and quality and may cause clogging of the plate. Read "Determining the amount of starting material" (page 15).
- If working with RNA for the first time, read Appendix C (page 53).
- 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 3.
  Homogenized cell lysates from step 4 can be stored at –70°C for several months. To process frozen homogenized lysates, incubate at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity.
- Generally, DNase digestion is not required since the combined QIAzol and RNeasy technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA. In these cases, small residual amounts of DNA can be removed by on-plate DNase digestion (see Appendix B, page 49) or by DNase digestion after RNA purification (please contact QIAGEN Technical Service for a protocol).
- Buffer RWT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature (15–25°C).
- QIAzol Lysis Reagent and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Use of a multichannel pipet is recommended (see page 10). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package.
- A vacuum source capable of generating a vacuum pressure of –800 to –900 mbar is necessary (see pages 13–16). The vacuum must be switched off and the manifold ventilated between pipetting steps to maintain uniform conditions for each sample.

- All centrifugation steps in the protocol are performed in a Centrifuge 4–16K (see page 11).
- The preliminary centrifugation step (step 6) and the centrifugation step to separate the aqueous from the organic phase (step 9) should be carried out at 4°C. All other steps of the miRNeasy 96 protocol should be performed at room temperature (15–25°C). Avoid interruptions during the procedure.

### Things to do before starting

- Buffer RWT and Buffer RPE are supplied as concentrates. Before using for the first time, add the required volumes of ethanol (96%–100%), as indicated on the bottle, to obtain a working solution.
- Check that all the buffers are at room temperature (15–25°C). If using the optional on-plate DNase digestion, see Appendix B (page 49) for details to prepare the DNase I incubation mix.

### Procedure

1. **Preparation of QIAvac 96 vacuum manifold: Place the waste tray inside the QIAvac base. Place the top plate squarely over the base. Place the RNeasy 96 plate in the QIAvac top plate, making sure that the plate is seated tightly. Attach the QIAvac 96 manifold to the vacuum source. Keep vacuum switched off.**

   **Note**: Always place the RNeasy 96 plate into the vacuum manifold with the beveled edges pointing to the right-hand side.

2. **Harvest cells according to step 2a. or 2b.**

**2a. Cells grown in a monolayer:**
**Cells grown in a monolayer in multiwell cell-culture plates can be lysed directly in the wells (do not use more than 1 x $10^7$ cells per well). Completely remove medium by pipetting, and continue with step 3.**

**Note**: Incomplete removal of the supernatant will dilute the QIAzol Lysis Reagent. This may lead to reduced RNA yield.

**2b. Cells grown in suspension:**
**Transfer aliquots of up to 1 x $10^7$ cells into collection microtubes (supplied). Spin cells for 5 min at 300 x *g*. Completely remove supernatant by pipetting and continue with step 3.**

**Note:** Incomplete removal of the supernatant will dilute the QIAzol Lysis Reagent. This may lead to reduced RNA yield.

3. **Disrupt the cells by adding QIAzol Lysis Reagent.**
   **For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add 700 µl QIAzol Lysis Reagent to each plate well/collection microtube. Pipet up and down 3 times.**
   **If the lysates are in plate wells, transfer to collection microtubes (supplied).**
4. **Close the collection microtubes using collection microtube caps (supplied). Vortex for 1 min at maximum speed.**

   Vortexing for 1 min is sufficient for homogenization of up to 3 x $10^6$ cells. If the cell number is greater than 3 x $10^6$, the TissueLyser II should be used to homogenize cells (as described in the protocols for animal tissue).

   **Note**: Homogenized cell lysates can be stored at –70°C for several months.
5. **Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.**

   This step promotes dissociation of nucleoprotein complexes.
6. **Centrifuge at 6000 x *g* for 1 min at 4°C to collect residual liquid from the caps of the tubes.**
7. **Add 140 µl chloroform. Securely cap the collection microtube rack containing the homogenates using new strips of collection microtube caps. Shake the collection microtube rack vigorously for 15 s.**

   Thorough mixing is important for subsequent phase separation.
8. **Place the collection microtube rack on the benchtop at room temperature (15–25°C) for 2–3 min.**
9. **Centrifuge at 6000 x *g* for 15 min at 4°C. After centrifuging, heat the centrifuge to room temperature if the same centrifuge will be used for the next centrifugation steps.**

   After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. The volume of the aqueous phase should be approximately 350 µl.

   **Note**: If you would like to purify a separate miRNA-enriched fraction, follow the steps in Appendix A (page 45) after performing this step.
10. **Transfer the upper aqueous phases to a new S-Block. Add 1.5 volumes (usually 525 µl) of 100% ethanol and mix by pipetting up and down. Do not centrifuge. Continue without delay with step 11.**

**11. Pipet the samples (approximately 875 µl) into the wells of the RNeasy 96 plate and switch on the vacuum source. Apply vacuum until transfer is complete (1–5 min). Switch off the vacuum and ventilate the QIAvac 96 manifold.**

Make sure that the QIAvac 96 vacuum manifold is assembled correctly before loading. The flow-through is collected in the waste tray. The vacuum must be switched off and the manifold ventilated between pipetting steps to maintain uniform conditions for each sample.

Take care not to wet the rims of the wells to avoid cross-contamination in subsequent steps

**Note**: Tape unused wells with adhesive tape. Do not use the AirPore Tape Sheets supplied with the miRNeasy 96 Kit. Use either adhesive tape or tape pads (cat. no. 19570) from QIAGEN.

**Optional DNase digestion**: If performing optional on-plate DNase digestion (see "Important points before starting"), follow steps B1–B4 (page 49) after performing this step.

**12. Add 800 µl Buffer RWT to each well of the RNeasy 96 plate. Switch on the vacuum source and apply vacuum until transfer is complete (1–5 min). Switch off the vacuum and ventilate the QIAvac 96 manifold.**

Collect the wash fraction in the same waste tray used in step 11.

Skip this step if performing the optional on-plate DNase digestion (page 49).

**13. Lift the top plate carrying the RNeasy 96 plate off the base, and empty the waste tray.* Reassemble the QIAvac 96 vacuum manifold.**

**14. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate and switch on the vacuum source. Apply vacuum until transfer is complete (1–5 min). Switch off the vacuum and ventilate the QIAvac 96 manifold.**

**15. Add another 800 µl Buffer RPE to each well of the RNeasy 96 plate and switch on the vacuum source. Apply vacuum until transfer is complete (1–5 min). Switch off the vacuum and ventilate the QIAvac 96 manifold.**

* Flow-through contains QIAzol Lysis Reagent and Buffer RWT and is therefore not compatible with bleach. See page 5 for safety information.

16. **Place the RNeasy 96 plate on top of an S-Block. Seal the RNeasy 96 plate with an AirPore Tape Sheet. Load the S-Block and RNeasy 96 plate into the holder and place the whole assembly in the rotor bucket. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 10 min at room temperature to dry the plate membranes.**

    Centrifugation with sealed plates prevents cross-contamination.

    It is important to dry the RNeasy membrane since residual ethanol may interfere with subsequent reactions. The 10 min spin ensures that residual traces of salt are removed and that no ethanol is carried over during elution.

17. **Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing elution microtubes.**

18. **To elute the RNA, add 45–70 µl RNase-free water to each well and seal the RNeasy 96 plate with a new AirPore Tape Sheet. Incubate for 1 min at room temperature. Then centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

    **Note**: Make sure to pipet the RNase-free water directly onto the RNeasy membrane. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.

19. **Remove the AirPore Tape Sheet. Repeat the elution step (step 18) with a second volume of 45–70 µl RNase-free water.**

    **Note**: Repeating the elution step is required for complete recovery of RNA.

    Use elution microtube caps provided to seal the microtubes for storage. Store RNA at –15 to –30°C or at –70°C.

# Protocol: Purification of Total RNA, Including Small RNAs, from Animal Cells using Spin Technology

### Important points before starting

- If using the miRNeasy 96 Kit for the first time, read "Important Notes" (page 15).
- It is important not to overload the RNeasy plate, as overloading will significantly reduce RNA yield and quality and may cause clogging of the plate. Read "Determining the amount of starting material" (page 15).
- If working with RNA for the first time, read Appendix C (page 53).
- 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. To process frozen homogenized lysates, incubate at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity.
- Generally, DNase digestion is not required since the combined QIAzol and RNeasy technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA. In these cases, small residual amounts of DNA can be removed by on-plate DNase digestion (see Appendix B, page 49) or by DNase digestion after RNA purification (please contact QIAGEN Technical Service for a protocol).
- Buffer RWT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature (15–25°C).
- QIAzol Lysis Reagent and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Use of a multichannel pipet is recommended (see page 10). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package.
- All centrifugation steps in the protocol are performed in a Centrifuge 4–16K (see page 11).
- The preliminary centrifugation step (step 5) and the centrifugation step to separate the aqueous from the organic phase (step 8) should be carried out at 4°C. All other steps of the miRNeasy 96 protocol should be performed at room temperature (15–25°C). Avoid interruptions during the procedure.

### Things to do before starting

- Buffer RWT and Buffer RPE are supplied as concentrates. Before using for the first time, add the required volumes of ethanol (96%–100%), as indicated on the bottle, to obtain a working solution.
- Check that all the buffers are at room temperature (15–25°C). If using the optional on-plate DNase digestion, see Appendix B (page 49) for details to prepare the DNase I incubation mix.

### Procedure

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

**1a. Cells grown in a monolayer:**
**Cells grown in a monolayer in multiwell cell-culture plates can be lysed directly in the wells (do not use more than 1 x $10^7$ cells per well). Completely remove medium by pipetting and continue with step 2.**

**Note**: Incomplete removal of the supernatant will dilute the QIAzol Lysis Reagent. This may lead to reduced RNA yield.

**1b. Cells grown in suspension:**
**Transfer aliquots of up to 1 x $10^7$ cells into collection microtubes (supplied). Spin cells for 5 min at 300 x *g*. Completely remove supernatant by pipetting and continue with step 2.**

**Note:** Incomplete removal of the supernatant will dilute the QIAzol Lysis Reagent. This may lead to reduced RNA yield.

**2. Disrupt the cells by adding QIAzol Lysis Reagent.**
**For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add 700 µl QIAzol Lysis Reagent to each plate well/collection microtube. Pipet up and down 3 times. If the lysates are in plate wells, transfer to collection microtubes (supplied).**

**3. Close the collection microtubes using collection microtube caps (supplied). Vortex for 1 min at maximum speed.**

Vortexing for 1 min is sufficient for homogenization of up to 3 x $10^6$ cells. If the cell number is greater than 3 x $10^6$, the TissueLyser II should be used to homogenize cells (as described in the protocols for animal tissue).

**Note**: Homogenized cell lysates can be stored at –70°C for several months.

**4. Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.**

This step promotes dissociation of nucleoprotein complexes.

**5. Centrifuge at 6000 x *g* for 1 min at 4°C to collect residual liquid from the caps of the tubes.**

6. **Add 140 µl chloroform. Securely cap the collection microtube rack containing the homogenates using new strips of collection microtube caps. Shake the collection microtube rack vigorously for 15 s.**

   Thorough mixing is important for subsequent phase separation.

7. **Place the collection microtube rack on the benchtop at room temperature for 2–3 min.**

8. **Centrifuge at 6000 x *g* for 15 min at 4°C. After centrifuging, heat the centrifuge to room temperature if the same centrifuge will be used for the next centrifugation steps.**

   After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. The volume of the aqueous phase should be approximately 350 µl.

   **Note**: If you want to purify a separate miRNA-enriched fraction, follow the steps in Appendix A (page 45) after performing this step.

9. **Transfer the upper aqueous phases to a new S-Block. Add 1.5 volumes (usually 525 µl) of 100% ethanol and mix by pipetting up and down. Do not centrifuge. Continue without delay with step 10.**

10. **Place an RNeasy 96 plate on top of an S-Block.**

11. **Pipet the samples (approximately 875 µl) from step 9 into the wells of the RNeasy 96 plate.**

    Take care not to wet the rims of the wells to avoid cross-contamination in subsequent steps.

12. **Seal the RNeasy 96 plate with an AirPore Tape Sheet. Load the S-Block and RNeasy 96 plate into the holder and place the whole assembly in the rotor bucket. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

    Centrifugation with sealed plates prevents cross-contamination.

    **Optional DNase digestion**: If performing optional on-plate DNase digestion (see "Important points before starting"), follow steps B1–B4 (page 49) after performing this step.

13. **Empty the S-Block* and remove the AirPore Tape Sheet. Add 800 µl Buffer RWT to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

    Skip this step if performing the optional on-plate DNase digestion (page 49).

* Flow-through contains QIAzol Lysis Reagent and is therefore not compatible with bleach. See page 5 for safety information.

14. **Empty the S-Block* and remove the AirPore Tape Sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

15. **Empty the S-Block and remove the AirPore Tape Sheet. Add another 800 µl Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 10 min at room temperature.**

    It is important to dry the RNeasy membrane since residual ethanol may interfere with subsequent reactions. The 10 min spin ensures that residual traces of salt are removed and that no ethanol is carried over during elution.

16. **Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing elution microtubes.**

17. **To elute the RNA, add 45–70 µl of RNase-free water to each well and seal the RNeasy 96 plate with a new AirPore Tape Sheet. Incubate for 1 min at room temperature. Then centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

    **Note**: Make sure to pipet the RNase-free water directly onto the RNeasy membrane. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.

18. **Remove the AirPore Tape Sheet. Repeat the elution step (step 17) with a second volume of 45–70 µl RNase-free water.**

    **Note**: Repeating the elution step is required for complete recovery of RNA.

    Use elution microtube caps provided to seal the microtubes for storage. Store RNA at –15 to –30°C or at –70°C.

* Flow-through contains Buffer RWT and is therefore not compatible with bleach. See page 5 for safety information.

# Protocol: Purification of Total RNA, Including Small RNAs, from Animal Tissues using Vacuum/Spin Technology

### Important points before starting

- If using the miRNeasy 96 Kit for the first time, read "Important Notes" (page 15).
- It is important not to overload the RNeasy plate, as overloading will significantly reduce RNA yield and quality and may cause clogging of the plate. Read "Determining the amount of starting material" (page 15).
- If preparing RNA for the first time, read Appendix C (page 53).
- Flash-frozen or RNA*later* stabilized tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling prior to disruption in QIAzol Lysis Reagent. Homogenized tissue lysates can also be stored at –70°C for several months. To process frozen homogenized lysates, thaw samples at room temperature or at 37°C in a water bath until they are completely thawed and salts in the QIAzol Lysis Reagent are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 9.
- Generally, DNase digestion is not required since the combined QIAzol and RNeasy technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. In these cases, small residual amounts of DNA can be removed by on-plate DNase digestion (see Appendix B, page 49) or by DNase digestion after RNA purification (please contact QIAGEN Technical Service for a protocol).
- Buffer RWT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature (15–25°C).
- QIAzol Lysis Reagent and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Use of a multichannel pipet is recommended (see page 10). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package.
- A vacuum source capable of generating a vacuum pressure of –800 to –900 mbar is necessary (see pages 13–16). The vacuum must be switched off and the manifold ventilated between pipetting steps to maintain uniform conditions for each sample.

- All centrifugation steps in the protocol are performed in a Centrifuge 4–16K (see page 11).
- The preliminary centrifugation step (step 9) and the centrifugation step to separate the aqueous from the organic phase (step 12) should be done at 4°C. All other steps of the miRNeasy 96 protocol should be performed at room temperature (15–25°C). Avoid interruptions during the procedure.

### Things to do before starting

- Buffer RWT and Buffer RPE are supplied as concentrates. Before using for the first time, add the required volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Check that all the buffers are at room temperature (15–25°C). If using the optional on-plate DNase digestion, see Appendix B (page 49) for details to prepare the DNase I incubation mix.

### Procedure

1. **Preparation of QIAvac 96 vacuum manifold: Place the waste tray inside the QIAvac base. Place the top plate squarely over the base. Place the RNeasy 96 plate in the QIAvac top plate, making sure that the plate is seated tightly. Attach the QIAvac 96 manifold to the vacuum source. Keep vacuum switched off.**

   **Note**: Always place the RNeasy 96 plate into the vacuum manifold with the beveled edges pointing to the right-hand side.

2. **Place 5 mm stainless steel beads into the collection microtubes (1 bead per tube), and transfer the collection microtube rack to a box with dry ice.**

   **Note**: We recommend using the TissueLyser II for disruption and homogenization of up to 192 samples in parallel. Alternatively, a rotor–stator homogenizer can be used for tissue disruption and homogenization. This protocol describes RNA purification from flash-frozen tissue using the TissueLyser II. For RNA*later* stabilized tissue, cooling the collection microtube rack on dry ice is not necessary.

3. **Excise the tissue sample from the animal or remove it from storage.**

   Do not allow unstabilized tissue to thaw before it is placed in QIAzol Lysis Reagent.

4. **Determine the amount of tissue. Do not use more than 50 mg flash-frozen tissue, 25 mg liver, thymus, spleen, or RNA*later* stabilized tissue, or 100 mg adipose tissue. Transfer it immediately to a cooled collection microtube. Repeat this until all required pieces of tissues are placed in the collection microtubes.**

   See page 15 for guidelines to determine the amount of starting material.

   RNA in unstabilized tissues is not protected after harvesting until the sample is flash-frozen or disrupted and homogenized in protocol steps 6 and 7. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

5. **Remove the collection microtube rack from the dry ice and immediately pipet 700 µl QIAzol Lysis Reagent into each collection microtube.**
6. **Close the collection microtube rack using the supplied strips of collection microtube caps and homogenize on the TissueLyser II for 5 min at 25 Hz.**
7. **Rotate the TissueLyser rack to allow even homogenization and homogenize for another 5 min at 25 Hz.**

   Some exceptionally tough tissues (e.g., pig skin) may not be completely homogenized after 2 x 5 min. This does not affect the protocol, however, since undisrupted pieces of tissue are removed after phase separation.

   **Note**: Homogenized tissue lysates can be stored at –70°C for several months.

8. **Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.**

   This step promotes dissociation of nucleoprotein complexes.

9. **Centrifuge at 6000 x *g* for 1 min at 4°C to collect residual liquid from the caps of the tubes.**
10. **Add 140 µl chloroform. Securely cap the collection microtube rack containing the homogenates using new strips of collection microtube caps. Shake the collection microtube rack vigorously for 15 s.**

    Thorough mixing is important for subsequent phase separation.

11. **Place the collection microtube rack on the benchtop at room temperature for 2–3 min.**

12. **Centrifuge at 6000 x *g* for 15 min at 4°C. After centrifuging, heat the centrifuge to room temperature if the same centrifuge will be used for the next centrifugation steps.**

    After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. For tissues with an especially high fat content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 350 µl.

    **Note**: If you want to purify a separate miRNA-enriched fraction, follow the steps in Appendix A (page 45) after performing this step.

13. **Transfer the upper aqueous phases to a new S-Block. Add 1.5 volumes (usually 525 µl) of 100% ethanol and mix by pipetting up and down. Do not centrifuge. Continue without delay with step 14.**

14. **Pipet the samples (approximately 875 µl) into the wells of the RNeasy 96 plate and switch on the vacuum source. Apply vacuum until transfer is complete (1–5 min). Switch off the vacuum and ventilate the QIAvac 96 manifold.**

    Make sure that the QIAvac 96 vacuum manifold is assembled correctly before loading. The flow-through is collected in the waste tray. The vacuum must be switched off and the manifold ventilated between pipetting steps to maintain uniform conditions for each sample.

    Take care not to wet the rims of the wells to avoid cross-contamination in subsequent steps

    **Note**: Tape unused wells with adhesive tape. Do not use the AirPore Tape Sheets supplied with the miRNeasy 96 Kit. Use either adhesive tape or tape pads (cat. no. 19570) from QIAGEN.

    **Optional DNase digestion**: If performing optional on-plate DNase digestion (see "Important points before starting"), follow steps B1–B4 (page 49) after performing this step.

15. **Add 800 µl Buffer RWT to each well of the RNeasy 96 plate. Switch on the vacuum source and apply vacuum until transfer is complete (1–5 min). Switch off the vacuum and ventilate the QIAvac 96 manifold.**

    Collect the wash fraction in the same waste tray used in step 14.

    Skip this step if performing the optional on-plate DNase digestion (page 49).

16. **Lift the top plate carrying the RNeasy 96 plate off the base and empty the waste tray.* Reassemble the QIAvac 96 vacuum manifold.**
17. **Add 800 µl Buffer RPE to each well of the RNeasy 96 plate and switch on the vacuum source. Apply vacuum until transfer is complete (1–5 min). Switch off the vacuum and ventilate the QIAvac 96 manifold.**
18. **Add another 800 µl Buffer RPE to each well of the RNeasy 96 plate and switch on the vacuum source. Apply vacuum until transfer is complete (1–5 min). Switch off the vacuum and ventilate the QIAvac 96 manifold.**
19. **Place the RNeasy 96 plate on top of an S-Block. Seal the RNeasy 96 plate with an AirPore Tape Sheet. Load the S-Block and RNeasy 96 plate into the holder and place the whole assembly in the rotor bucket. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 10 min at room temperature to dry the plate membranes.**

    Centrifugation with sealed plates prevents cross-contamination.

    It is important to dry the RNeasy membrane since residual ethanol may interfere with subsequent reactions. The 10 min spin ensures that residual traces of salt are removed and that no ethanol is carried over during elution.
20. **Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing elution microtubes.**
21. **To elute the RNA, add 45–70 µl RNase-free water to each well and seal the RNeasy 96 plate with a new AirPore Tape Sheet. Incubate for 1 min at room temperature. Then centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

    **Note**: Make sure to pipet the RNase-free water directly onto the RNeasy membrane. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.
22. **Remove the AirPore Tape Sheet. Repeat the elution step (step 21) once with a second volume of 45–70 µl RNase-free water.**

    **Note**: Repeating the elution step is required for complete recovery of RNA.

    Use elution microtube caps provided to seal the microtubes for storage. Store RNA at –15 to –30°C or at –70°C.

* Flow-through contains QIAzol Lysis Reagent and Buffer RWT and is therefore not compatible with bleach. See page 5 for safety information.

# Protocol: Purification of Total RNA, Including Small RNAs, from Animal Tissues using Spin Technology

### Important points before starting

- If using the miRNeasy 96 Kit for the first time, read "Important Notes" (page 15).
- It is important not to overload the RNeasy plate, as overloading will significantly reduce RNA yield and quality and may cause clogging of the plate. Read "Determining the amount of starting material" (page 15).
- If preparing RNA for the first time, read Appendix C (page 53).
- Flash-frozen or RNA*later* stabilized tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling prior to disruption in QIAzol Lysis Reagent. Homogenized tissue lysates can also be stored at –70°C for several months. To process frozen homogenized lysates, thaw samples at room temperature or at 37°C in a water bath until they are completely thawed and salts in the QIAzol Lysis Reagent are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 8.
- Generally, DNase digestion is not required since the combined QIAzol and RNeasy technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. In these cases, small residual amounts of DNA can be removed using by on-plate DNase digestion (see Appendix B, page 49) or by DNase digestion after RNA purification (please contact QIAGEN Technical Service for a protocol).
- Buffer RWT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature (15–25°C).
- QIAzol Lysis Reagent and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Use of a multichannel pipet is recommended (see page 10). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package.
- All centrifugation steps in the protocol are performed in a Centrifuge 4–16K (see page 11).

- The preliminary centrifugation step (step 8) and the centrifugation step to separate the aqueous from the organic phase (step 11) should be done at 4°C. All other steps of the miRNeasy 96 protocol should be performed at room temperature (15–25°C). Avoid interruptions during the procedure.

### Things to do before starting

- Buffer RWT and Buffer RPE are supplied as concentrates. Before using for the first time, add the required volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Check that all the buffers are at room temperature (15–25°C). If using the optional on-plate DNase digestion, see Appendix B (page 49) for details to prepare the DNase I incubation mix.

### Procedure

1. **Place 5 mm stainless steel beads into the collection microtubes (1 bead per tube), and transfer the collection microtube rack to a box with dry ice.**

   **Note**: We recommend using the TissueLyser II for disruption and homogenization of up 192 samples in parallel. Alternatively, a rotor–stator homogenizer can be used for tissue disruption and homogenization. This protocol describes RNA purification from flash-frozen tissue using the TissueLyser II. For RNA*later* stabilized tissue, cooling the collection microtube rack on dry ice is not necessary.

2. **Excise the tissue sample from the animal or remove it from storage.**

   Do not allow unstabilized tissue to thaw before it is placed in QIAzol Lysis Reagent.

3. **Determine the amount of tissue. Do not use more than 50 mg flash-frozen tissue, 25 mg liver, thymus, spleen, or RNA*later* stabilized tissue, or 100 mg adipose tissue. Transfer it immediately to a cooled collection microtube. Repeat this until all required pieces of tissues are placed in the collection microtubes.**

   See page 15 for guidelines to determine the amount of starting material.

   RNA in unstabilized tissues is not protected after harvesting until the sample is flash-frozen, or disrupted and homogenized in protocol steps 5 and 6. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

4. **Remove the collection microtube rack from the dry ice and immediately pipet 700 µl QIAzol Lysis Reagent into each collection microtube.**

5. **Close the collection microtube rack using the supplied strips of collection microtube caps and homogenize on the TissueLyser II for 5 min at 25 Hz.**
6. **Rotate the TissueLyser rack to allow even homogenization and homogenize for another 5 min at 25 Hz.**

   Some exceptionally tough tissues (e.g., pig skin) may not be completely homogenized after 2 x 5 min. This does not affect the protocol, however, since undisrupted pieces of tissue are removed after phase separation.

   **Note**: Homogenized tissue lysates can be stored at –70°C for several months.
7. **Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.**

   This step promotes dissociation of nucleoprotein complexes.
8. **Centrifuge at 6000 x *g* for 1 min at 4°C to collect residual liquid from the caps of the tubes.**
9. **Add 140 µl chloroform. Securely cap the collection microtube rack containing the homogenates using new strips of caps and shake it vigorously for 15 s.**

   Thorough mixing is important for subsequent phase separation.
10. **Place the collection microtube rack on the benchtop at room temperature for 2–3 min.**
11. **Centrifuge at 6000 x *g* for 15 min at 4°C. After centrifuging, heat the centrifuge to room temperature if the same centrifuge is to be used for the next centrifugation steps.**

    After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. For tissues with an especially high fat content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 350 µl.

    **Note**: If you want to purify a separate miRNA-enriched fraction, follow the steps in Appendix A (page 45) after performing this step.
12. **Transfer the upper aqueous phases to a new S-Block. Add 1.5 volumes (usually 525 µl) of 100% ethanol and mix by pipetting up and down. Do not centrifuge. Continue without delay with step 13.**
13. **Place an RNeasy 96 plate on top of an S-Block.**
14. **Pipet the samples (approximately 875 µl) from step 12 into the wells of the RNeasy 96 plate.**

    Take care not to wet the rims of the wells to avoid cross-contamination in subsequent steps.

15. **Seal the RNeasy 96 plate with an AirPore Tape Sheet. Load the S-Block and RNeasy 96 plate into the holder and place the whole assembly in the rotor bucket. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

    Centrifugation with sealed plates prevents cross-contamination.

    **Optional DNase digestion**: If performing optional on-plate DNase digestion (see "Important points before starting"), follow steps B1–B4 (page 49) after performing this step.

16. **Empty the S-Block* and remove the AirPore Tape Sheet. Add 800 µl Buffer RWT to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

    Skip this step if performing the optional on-plate DNase digestion (page 49).

17. **Empty the S-Block* and remove the AirPore Tape Sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

18. **Empty the S-Block and remove the AirPore Tape Sheet. Add another 800 µl Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 10 min at room temperature.**

    It is important to dry the RNeasy membrane since residual ethanol may interfere with subsequent reactions. The 10 min spin ensures that residual traces of salt are removed and that no ethanol is carried over during elution.

19. **Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing elution microtubes.**

20. **To elute the RNA, add 45–70 µl of RNase-free water to each well and seal the RNeasy 96 plate with a new AirPore Tape Sheet. Incubate for 1 min at room temperature. Then centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

    **Note**: Make sure to pipet the RNase-free water directly onto the RNeasy membrane. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.

* Flow-through contains QIAzol Lysis Reagent or Buffer RWT and is therefore not compatible with bleach. See page 5 for safety information.

* Flow-through contains QIAzol Lysis Reagent or Buffer RWT and is therefore not compatible with bleach. See page 5 for safety information.

21. **Remove the AirPore Tape Sheet. Repeat the elution step (step 20) once with a second volume of 45–70 µl RNase-free water.**

**Note**: Repeating the elution step is required for complete recovery of RNA.

Use elution microtube caps provided to seal the microtubes for storage. Store RNA at –15 to –30°C or at –70°C.

# 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. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

| | Comments and suggestions |
|---|---|
| **Phases do not separate completely** | |
| a) No chloroform added or chloroform not pure | Make sure to add chloroform that does not contain isoamyl alcohol or other additives. |
| b) Homogenate not sufficiently mixed before centrifugation | After addition of chloroform, the homogenate must be vigorously shaken. If the phases are not well separated, shake the tube vigorously for at least 15 s and repeat the incubation and centrifugation. |
| c) Organic solvents in samples used for RNA purification | Make sure that the starting sample does not contain organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline reagents. These can interfere with the phase separation. |
| **Clogged plate wells** | |
| a) Too much starting material | In subsequent preparations, reduce the amounts of starting material. It is essential to use the correct amount of starting material (see page 15). |
| b) Inefficient disruption and/or homogenization | See "Disrupting and homogenizing starting materials" (pages 18–22) for a detailed description of homogenization methods.<br>Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see page 15) and/or increase the homogenization time. |

| | Comments and suggestions |
|---|---|
| c) Centrifugation temperature too low | Except for the preliminary centrifugation step and the phase separation step, all centrifugation steps should be performed at room temperature (15–25°C). Some centrifuges may cool to below 20°C even when set at 20°C. This can cause precipitates to form that can clog the RNeasy 96 plate. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol-containing lysate to 37°C before transferring to the RNeasy 96 plate. |
| **Little or no RNA eluted** | |
| a) Too much starting material | Overloading significantly reduces yield. Reduce the amount of starting material (see page 15). Make sure to use the ethanol concentrations specified in the protocol steps. |
| b) Inefficient disruption and/or homogenization | See "Disruption and homogenization of starting materials" (pages 18–22) for a detailed description of homogenization methods. |
| c) Buffer temperatures too low | All buffers must be at room temperature (15–25°C) throughout the procedure. |
| d) RNA still bound to the membrane | Repeat elution, but incubate the RNeasy 96 plate on the benchtop for 10 min with RNase-free water before centrifuging. |
| **Low miRNA yield or poor performance of miRNA in downstream experiments** | |
| a) Incorrect ethanol concentration | Be sure to use the ethanol concentrations specified in the protocol steps. |
| b) Interference from large RNAs | In some assays, the presence of mRNA and rRNA can result in increased background. In this case, follow the protocol in Appendix A (page 45) to isolate a separate, miRNA-enriched fraction. |
| **Low $A_{260}/A_{280}$ value** | |
| a) Not enough QIAzol Lysis Reagent used for homogenization | Reduce the amount of starting material and/or increase the volume of QIAzol Lysis Reagent and the homogenization time. |

| | Comments and suggestions |
|---|---|
| b) Sample not incubated for 5 min after homogenization | Place the sample at room temperature (15–25°C) for 5 min after homogenization, as indicated in the protocols. This step is important to promote dissociation of nucleoprotein complexes. |
| c) Water used to dilute RNA for $A_{260}/A_{280}$ measurement | Use 10 mM Tris·Cl,* pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see Appendix D, page 55). |
| **RNA degraded** | |
| a) Inappropriate handling of starting material | Ensure that tissues have been properly handled and that the protocol has been performed without interruptions, especially the initial steps involving tissue lysis and homogenization. Some tissues (e.g., pancreas or intestine) contain high amounts of RNases. Care must be taken to excise these tissue from animals as fast as possible and to stabilize them either by freezing in liquid nitrogen or by immersing them in RNA*later* RNA Stabilization Reagent† immediately after excision. |
| b) RNase contamination | Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Make sure not to introduce any RNases during the procedure or later handling. See "Appendix C: General Remarks on Handling RNA" (page 53). |
| **DNA contamination in downstream experiments** | |
| a) Phase separation performed at too high a temperature | The phase separation should be performed at 4°C to allow optimal phase separation and removal of genomic DNA from the aqueous phase. Make sure that the centrifuge does not heat above 10°C during the centrifugation. |

* 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.

† See the *RNA*later *Handbook* for more information about RNA*later* RNA Stabilization Reagent.

| | Comments and suggestions |
|---|---|
| b) Interphase contamination of aqueous phase | Contamination of the aqueous phase with the interphase results in increased DNA content in the eluate. Make sure to transfer the aqueous phase without interphase contamination. |
| c) No DNase treatment | Follow the optional on-plate DNase digestion using the RNase-Free DNase Set (Appendix B, page 49) at the point indicated in the protocol.<br><br>Alternatively, after the miRNeasy procedure, DNase digest the eluate containing the RNA. After inactivating DNase by heat treatment, the RNA can be either used directly in the subsequent application without further treatment, or repurified using an RNeasy RNA cleanup protocol (see the *RNeasy 96 Handbook*). |

**RNA concentration too low**

| | |
|---|---|
| Elution volume too high | Elute with less RNase-free water. Although eluting with less water results in increased RNA concentrations, total yields might be reduced. |

**RNA does not perform well in downstream experiments**

| | |
|---|---|
| a) Salt carryover during elution | Ensure that Buffer RPE is at room temperature (15–25°C). |
| b) Ethanol carryover | For spin protocols only: during the second Buffer RPE wash, be sure to dry the plate-well membranes by centrifugation at 6000 rpm (approximately 5600 x *g*) for 10 min at room temperature (15–25°C). |
| c) Vacuum/spin protocol: Vacuum pressure too low | A vacuum source capable of generating a vacuum pressure of –800 to –900 mbar is necessary to achieve efficient RNA binding and washing. |

**Low well-to-well reproducibility**

| | |
|---|---|
| a) Elution volume too low | Use elution volumes of 2 x 50 µl or 2 x 75 µl to improve well-to-well reproducibility. |
| b) Vacuum pressure too low | A vacuum source capable of generating a vacuum pressure of –800 to –900 mbar is necessary to achieve efficient RNA binding and washing. |

| | Comments and suggestions |
|---|---|
| c) Incomplete homogenization | Some types of tissues are more difficult to homogenize, resulting in greater variability from sample to sample. |
| d) Variability between samples | RNA yields from tissue samples can vary more than cultured cells due to the heterogeneous nature of most tissues and donor-to-donor variability. |

# Appendix A: Preparation of miRNA-Enriched Fractions Separate from Larger RNAs (>200 nt)

This protocol allows purification of a separate fraction, enriched in miRNA and other small RNA species. Removal of larger RNAs, such as mRNA and rRNA, may reduce background in certain downstream applications.

For this protocol, an additional RNeasy 96 plate is required. This will reduce the number of possible preparations from one miRNeasy 96 Kit from 4 x 96 to 2 x 96. As an economical alternative, we recommend purchase of an RNeasy 96 Kit (for ordering information, see page 58).

### Quantification of miRNA

The miRNA-enriched fraction obtained using this protocol is enriched in various RNAs of <200 nucleotides (e.g., tRNAs). For this reason, the miRNA yield cannot be quantified by OD measurement or fluorogenic assays. To determine yield, we recommend using quantitative, real-time RT-PCR assays specific for the type of small RNA under study. For example, to estimate miRNA yield, an assay directed against any miRNA known to be adequately expressed in the samples being processed may be used.

### Procedure

Carry out all protocol steps to phase separation. Instead of continuing with the next step of the protocol, follow steps A1–A15 below to isolate the miRNA-enriched fraction only or steps A1–A22 to isolate separate fractions of small RNA and total RNA >200 nt.

**Note**: If the vacuum/spin protocol is used, centrifugation in steps A8–A12 and A17–A19 can be replaced by use of the vacuum. In this case, plates should be dried by centrifuging at 6000 rpm for 10 min prior to elution to avoid carryover of trace amounts of ethanol.

**A1. Transfer the upper aqueous phases to a new S-Block. Add 1 volume of 70% ethanol (usually 350 µl) and mix by pipetting up and down. Do not centrifuge. Proceed immediately to step A2.**

**A2. Place the RNeasy 96 plate on top of a new S-Block.**

**A3. Pipet the samples (approx. 700 µl), including any precipitates that may have formed, into the wells of the RNeasy 96 plate.**

**A4. Seal the RNeasy 96 plate with an AirPore Tape Sheet. Load the S-Block and RNeasy 96 plate into the holder and place the whole assembly in the rotor bucket. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature (15–25°C).**

Keep the S-Block with the flow-through for later purification of the miRNA-enriched fraction.

**A5. If purifying the miRNA-enriched fraction only, discard the RNeasy 96 plate and follow steps A6–A15 only.**

**If purifying both miRNA-enriched fraction and larger RNAs (>200 nt), save the RNeasy 96 plate for use in step A16 (the plate can be stored at 4°C or at room temperature [15–25°C], but not for long periods). Follow steps A6–A15 to purify miRNA and then steps A16–A22 to purify large RNAs.**

**Purifying the miRNA-enriched fraction**

**A6. Add 0.65 volumes of 100% ethanol (usually 450 µl) to the S-Block containing the flow-through from step A4 and mix by pipetting up and down. Do not centrifuge. Proceed immediately to step A7.**

**A7. Place a new RNeasy 96 plate on top of an S-Block.**

**A8. Pipet 900 µl of each sample into the wells of the new RNeasy 96 plate. Seal the RNeasy 96 plate with an AirPore Tape Sheet. Load the S-Block and RNeasy 96 plate into the holder, and place the whole assembly in the rotor bucket. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature (15–25°C).**

**A9. Empty the S-Block,* remove the AirPore Tape Sheet, and repeat step A8 with the remaining sample.**

**A10. Optional: Empty the S-Block* and remove the AirPore Tape Sheet. Add 800 µl Buffer RWT to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

Do not perform this step if you are purifying both the miRNA-enriched fraction and larger RNAs (>200 nt).

**A11. Empty the S-Block* and remove the AirPore Tape Sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

**A12. Empty the S-Block and remove the AirPore Tape Sheet. Add another 800 µl Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 10 min at room temperature.**

It is important to dry the RNeasy membrane since residual ethanol may interfere with subsequent reactions. The 10 min spin ensures that residual traces of salt are removed and that no ethanol is carried over during elution.

* Flow-through contains QIAzol Lysis Reagent or Buffer RWT and is therefore not compatible with bleach. See page 5 for safety information.

**A13. Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing elution microtubes.**

**A14. To elute the small RNA fraction, add 45–70 µl of RNase-free water to each well and seal the RNeasy 96 plate with a new AirPore Tape Sheet. Incubate for 1 min at room temperature. Then centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

**A15. Remove the AirPore Tape Sheet. Repeat the elution step (step A14) with a second volume of 45–70 µl RNase-free water.**

**Note**: Repeating the elution step is required for complete recovery of RNA.

**Purifying total RNA (>200 nt)**

**A16. Place the RNeasy 96 plate from step A5 on top of an S-Block.**

**A17. Add 800 µl Buffer RWT to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

**Optional**: If on-plate DNase digestion using RNase-Free DNase Sets is desired, perform steps B1–B4 (Appendix B, page 49) instead of this step. Then proceed to step A18.

**A18. Empty the S-Block* and remove the AirPore Tape Sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.**

**A19. Empty the S-Block and remove the AirPore Tape Sheet. Add another 800 µl Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 10 min at room temperature.**

It is important to dry the RNeasy membrane since residual ethanol may interfere with subsequent reactions. The 10 min spin ensures that residual traces of salt are removed and that no ethanol is carried over during elution.

**A20. Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing elution microtubes.**

**A21. To elute the RNA, add 45–70 µl of RNase-free water to each well and seal the RNeasy 96 plate with a new AirPore Tape Sheet. Incubate for 1 min at room temperature. Then centrifuge at 6000 rpm (approximately 5600 x g) for 4 min at room temperature.**

* Flow-through contains Buffer RWT and is therefore not compatible with bleach. See page 5 for safety information.

**A22. Remove the AirPore Tape Sheet. Repeat the elution step with a second volume of 45–70 µl RNase-free water.**

**Note**: Repeating the elution step is required for complete recovery of RNA.

# Appendix B: Optional On-Plate DNase Digestion with the RNase-Free DNase Set

The RNase-Free DNase Set (cat. no. 79254) provides efficient on-plate digestion of DNA during RNA purification. The DNase is efficiently removed in subsequent wash steps. The miRNeasy 96 procedure requires 2 RNase-Free DNase Sets per 96-well plate.

**Note**: Buffer RDD supplied with the RNase-Free DNase Set is specially optimized for on-plate DNase digestion. However, to prevent losses of small RNAs, a modified DNase digestion procedure is recommended for samples containing less than approximately 1 µg total RNA (equivalent to about 1 x $10^5$ cells). In the modified procedure, the flow-through after on-plate digestion and washing is reapplied to the membrane. Buffer RWT used in this protocol should be prepared with isopropanol instead of ethanol, as is usually recommended (pages 22, 27, 31, and 36). Therefore, if not all preps will be performed using the procedure for DNase digestion for samples containing <1 µg total RNA approximately, it will be necessary to purchase additional Buffer RWT (cat. no. 1067933), which should be prepared with isopropanol.

For larger sample amounts containing greater than approximately 1 µg total RNA, recovery of tRNA may be reduced by DNase digestion, but miRNA yields are not affected.

Use of DNase buffers other than that supplied with the RNase-Free DNase Set may affect the binding of the RNA to the RNeasy plate, reducing the yield and integrity of the RNA.

### Important points before starting

- Generally, DNase digestion is not required since the integrated QIAzol and RNeasy technologies efficiently remove most of the DNA without DNase treatment. In addition, miScript Primer Assays and most other assays for mature miRNA are not affected by the presence of small amounts of genomic DNA. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., QuantiFast® analysis with a low-abundance target). Alternatively, DNA can be removed by a DNase digestion following RNA purification.
- DNase digestion is not necessary for miRNA-enriched fractions prepared using the protocol in Appendix A. This is because any residual DNA not removed in the organic extraction step will be retained together with larger RNAs by the first RNeasy 96 plate (step A2).
- **Do not vortex the reconstituted DNase I.** DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.

### Things to do before starting

- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. The miRNeasy 96 procedure requires 2 RNase-Free DNase Sets per 96-well plate. Dissolve 2 vials of lyophilized DNase I (1500 Kunitz units) in 2 x 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 –15 to –30°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.
- Carry out all protocol steps until the aqueous phase has been transferred through the RNeasy 96 plate (steps 1–11 of the animal cells-vacuum/spin protocol; steps 1–12 of the animal cells-spin protocol; steps 1–14 of the animal tissues-vacuum/spin protocol; steps 1–15 of the animal tissues-spin protocol; steps A1–A16 of the miRNA-enriched fraction protocol). Then follow steps B1–B4 below. If a vacuum/spin protocol is used, centrifugation in steps B1-B4 can be replaced by use of the vacuum. For the protocol for samples containing <1 µg total RNA approximately, prepare Buffer RWT by adding 45 ml isopropanol to the concentrate (instead of 30 ml ethanol as usually recommended). Buffer RWT can be ordered separately for this protocol (cat. no. 1067933).

### Procedure: DNase digestion for samples containing >1 µg total RNA approximately

Carry out all protocol steps until the aqueous phase has been transferred through the RNeasy 96 plate (steps 1–11 of the animal cells-vacuum/spin protocol; steps 1–12 of the animal cells-spin protocol; steps 1–14 of the animal tissues-vacuum/spin protocol; steps 1–15 of the animal tissues-spin protocol; steps A1–A16 of the miRNA-enriched fraction protocol). Then follow steps B1–B4 below. If a vacuum/spin protocol is used, centrifugation in steps B1-B4 can be replaced by use of the vacuum.

**B1. Pipet 400 µl Buffer RWT into each well of the RNeasy 96 plate and centrifuge for 4 min at 6000 rpm (approximately 5600 x *g*) to wash. Discard the flow-through.***

Reuse the S-Block in step B4.

* Flow-through contains Buffer RWT and is therefore not compatible with bleach. See page 5 for safety information.

**B2. Add 670 µl DNase I stock solution to 7.3 ml Buffer RDD. Mix by gently inverting the tube. Do not vortex.**

Buffer RDD is supplied with the RNase-Free DNase Set.

**B3. Pipet the DNase I incubation mix (80 µl per well) directly onto the membrane in each well of the RNeasy 96 plate and place on the benchtop at 20–30°C for 15 min.**

**Note:** Make sure to pipet the DNase I incubation mix directly onto the RNeasy membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the RNeasy 96 plate wells.

**B4. Pipet 400 µl Buffer RWT into each well of the RNeasy 96 plate and centrifuge for 4 min at 6000 rpm (approx. 5600 x *g*). Discard the flow-through.* Continue with the protocol (at step 13 of the animal cells-vacuum/spin protocol; step 14 of the animal cells-spin protocol; step 16 of the animal tissues-vacuum/spin protocol; step 17 of the animal tissues-spin protocol; step A18 of the miRNA-enriched fraction protocol).**

**Procedure: DNase digestion for samples containing <1 µg total RNA approximately**

Carry out all protocol steps until the aqueous phase has been transferred through the RNeasy 96 plate (steps 1–11 of the animal cells-vacuum/spin protocol; steps 1–12 of the animal cells-spin protocol; steps 1–14 of the animal tissues-vacuum/spin protocol; steps 1–15 of the animal tissues-spin protocol; steps A1–A5 of the miRNA-enriched fraction protocol).. Then follow steps B1–B6 below.

**B1. Pipet 350 µl Buffer RWT (prepared with isopropanol) into the RNeasy 96 plate and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm) to wash. Discard the flow-through.***

Reuse the collection tube in step B4.

**B2. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube. Do not vortex.**

Buffer RDD is supplied with the RNase-Free DNase Set.

* Flow-through contains Buffer RWT and is therefore not compatible with bleach. See page 5 for safety information.

**B3. Pipet the DNase I incubation mix (80 µl) directly onto the RNeasy 96 plate membrane and place on the benchtop at 20–30°C for 15 min.**

**Note**: Make sure to pipet the DNase I incubation mix directly onto the RNeasy membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the RNeasy 96 plate.

**B4. Pipet 500 µl Buffer RWT (prepared with isopropanol) into the RNeasy 96 plate and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Keep the flow-through.**

**B5. Reapply the flow-through to the RNeasy 96 plate and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.***

**B6. Continue with step 12 of the protocol or step A18 (if performing the protocol in Appendix A).**

# Appendix C: General Remarks on Handling RNA

## Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to degrade RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

## General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

To remove RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipets and electrophoresis tanks), use of RNaseKiller (cat. no. 2500080) from 5 PRIME (www.5prime.com) is recommended. RNase contamination can alternatively be removed using general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 54), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS),* rinse with RNase-free water, rinse with ethanol (if the tanks are ethanol-resistant), and allow to dry.

## Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate data sheets (SDSs), available from the product supplier.

## Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for 4 hours or more (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate), as described in "Solutions" below.

## Solutions

Solutions (water and other solutions)* should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and $CO_2$. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

**Note**: RNeasy buffers are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

* 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.

# Appendix D: Storage, Quantification, and Determination of Quality of RNA

## Storage of RNA

Purified RNA may be stored at –15 to –30°C or –70°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

## Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm ($A_{260}$) in a spectrophotometer (see "Spectrophotometric quantification of RNA" below). For small amounts of RNA, however, it may be difficult to determine amounts photometrically. Small amounts of RNA can be accurately quantified using an Agilent® 2100 Bioanalyzer®, quantitative RT-PCR, or fluorometric quantification. When purifying RNA from particularly small samples (e.g., laser-microdissected samples, or from plasma or serum), quantitative, real-time RT-PCR should be used for quantification.

## Spectrophotometric quantification of RNA

To ensure significance, $A_{260}$ readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml ($A_{260}=1\rightarrow$ 44 µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 56), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 54). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 100 µl

Dilution = 10 µl of RNA sample + 490 µl of 10 mM Tris·Cl,* pH 7.0 (1/50 dilution)

Measure absorbance of diluted sample in a 1 ml cuvette (RNase-free)

$A_{260}$ = 0.2

| | |
|---|---|
| Concentration of RNA sample | = 44 µg/ml x $A_{260}$ x dilution factor |
| | = 44 µg/ml x 0.2 x 50 |
| | = 440 µg/ml |
| Total amount | = concentration x volume in milliliters |
| | = 440 µg/ml x 0.1 ml |
| | = 44 µg of RNA |

### Purity of RNA

The ratio of the readings at 260 nm and 280 nm ($A_{260}/A_{280}$) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as protein. However, the $A_{260}/A_{280}$ ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting $A_{260}/A_{280}$ ratio can vary greatly. Lower pH results in a lower $A_{260}/A_{280}$ ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an $A_{260}/A_{280}$ ratio of 1.9–2.1† in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution.

For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration ($A_{260}$ reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Quantification of RNA", page 55).

### DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. While miRNeasy Kits will remove the vast majority of cellular DNA, trace amounts may still remain, depending on the amount and nature of the sample.

For analysis of very low-abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

To prevent any interference by DNA in gene expression analysis real-time RT-PCR applications, such as with ABI PRISM® and LightCycler® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect Primer Assays from QIAGEN are designed for SYBR® Green-based real-time RT-PCR analysis of RNA

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

† Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

sequences (without detection of genomic DNA) where possible (the assays can be ordered online at www.qiagen.com/GeneGlobe). For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, we recommend using the QuantiTect Reverse Transcription Kit for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination.

Alternatively, gene expression analysis can be performed using QuantiFast Probe Assays and the QuantiFast Probe RT-PCR Plus Kit, which includes an integrated genomic DNA removal step.

miScript Primer Assays, used with the miScript PCR System for miRNA quantification, do not detect genomic DNA.

For other sensitive applications, DNase digestion of the purified RNA with RNase-free DNase is recommended. A protocol for optional on-plate DNase digestion using the RNase-Free DNase Set is provided in Appendix B (page 49). The DNase is efficiently washed away in subsequent wash steps.

### Integrity of RNA

The integrity and size distribution of total RNA purified with miRNeasy Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide* staining or by using the QIAxcel® system or Agilent 2100 Bioanalyzer. The respective ribosomal RNAs should appear as sharp bands or peaks on the stained gel. The apparent ratio of 28S rRNA to 18S rRNA should be present at approximately 2:1. If the ribosomal bands or peaks of a given lane specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the RNA sample suffered major degradation either before or during RNA purification. The Agilent 2100 Bioanalyzer also provides an RNA Integrity Number (RIN) as a useful measure of RNA integrity. Ideally, the RIN should be close to 10, but in many cases (particularly with tissue samples), RNA quality is greatly influenced by how well the original sample was preserved.

* 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.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| miRNeasy 96 Kit (4) | For 4 x 96 preps: 4 RNeasy 96 plates, Collection Microtubes (racked), Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers | 217061 |
| **Related products** | | |
| miRNeasy Mini Kit (50) | For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-free Reagents and Buffers | 217004 |
| RNeasy 96 Kit (4)* | For 4 x 96 preps: 4 RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, RNase-Free Reagents and Buffers | 74181 |
| miRNeasy FFPE Kit (50) | 50 RNeasy MinElute® Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, DNase Booster Buffer, RNase-Free Buffers, RNase-Free Water | 217504 |
| miRNeasy Micro Kit (50) | For 50 total RNA preps: 50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-free Reagents and Buffers | 217084 |
| miRNeasy Serum/Plasma Kit (50) | For 50 total RNA preps: 50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-free Reagents and Buffers | 217184 |
| miRNeasy Serum/Plasma Spike-In Control | 10 pmol lyophilized *C. elegans* miR-39 miRNA mimic | 219610 |

* Other kit sizes are available; see www.qiagen.com.

| Product | Contents | Cat. no. |
|---|---|---|
| **Accessories** | | |
| TissueLyser II | Bead mill, 100-120/220-240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately) | 85300 |
| TissueLyser Adapter Set 2 x 96 | 2 sets of Adapter Plates for use with collection microtubes (racked) on the TissueLyser | 69984 |
| Stainless Steel Beads, 5 mm (200) | Stainless Steel Beads, suitable for use with the TissueLyser system | 69989 |
| TissueLyser 5 mm Bead Dispenser, 96-Well | For dispensing 96 beads (5 mm diameter) in parallel | 69975 |
| Centrifuge 4–16K | Refrigerated universal laboratory centrifuge with brushless motor | 81400*; 81410†; 81425‡; 81420§ |
| Plate Rotor 2 x 96 | Rotor for 2 QIAGEN 96 plates, for use with QIAGEN Centrifuges | 81031 |
| RNAprotect Cell Reagent (250 ml) | 250 ml RNAprotect Cell Reagent | 76526 |
| RNA*later* RNA Stabilization Reagent (50 ml)** | 50 ml RNA*later* RNA Stabilization Reagent for stabilization of RNA in animal tissues | 76104 |
| RNA*later* TissueProtect Tubes (50 x 1.5 ml) | For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNA*later* RNA Stabilization Reagent each | 76154 |

* Japan.

† North America.

‡ UK.

§ Rest of World.

** Other kit sizes are available; see www.qiagen.com.

| Product | Contents | Cat. no. |
|---|---|---|
| RNA*later* TissueProtect Tubes (20 x 5 ml) | For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNA*later* RNA Stabilization Reagent each | 76163 |
| QIAvac 96 | Vacuum manifold for processing QIAGEN 96-well plates: includes QIAvac 96 Top Plate, Base, Waste Tray, Plate Holder, Rack of Collection Microtubes (1.2 ml) | 19504 |
| Vacuum Regulator | For use with QIAvac manifolds | 19530 |
| Vacuum Pump (230 V, 50 Hz) | Universal vacuum pump (capacity 34 L/min, 8 mbar vacuum abs.) | 84020 |
| RNase-Free DNase Set (50) | 1500 units RNase-free DNase I, RNase-Free Buffer RDD, and RNase-Free Water for 50 RNA minipreps | 79254 |
| QIAzol® Lysis Reagent (200 ml) | 200 ml QIAzol Lysis Reagent | 79306 |
| S-Blocks (24) | 96-well blocks with 2.2 ml wells, 24 per case | 19585 |
| Elution Microtubes CL (24 x 96) | Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips | 19588 |
| Collection Microtubes (racked) | Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96 | 19560 |
| Collection Microtube Caps | Nonsterile polypropylene caps for collection microtubes (1.2 ml) and round-well blocks, 960 in strips of 8 | 19566 |
| Tape pads (5) | Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack | 19570 |
| AirPore Tape Sheets (50) | Microporous tape sheets for covering 96-well blocks: 50 sheets per pack | 19571 |

| Product | Contents | Cat. no. |
|---|---|---|
| **Related products for quantitative, real-time RT-PCR** | | |
| miScript II RT Kit (12) | For 12 cDNA synthesis reactions: miScript Reverse Transcriptase Mix, 10x miScript Nucleics Mix, 5x miScript HiSpec Buffer, 5x miScript HiFlex Buffer, RNase-Free Water | 218160 |
| miScript SYBR Green PCR Kit (200) | For 200 reactions: QuantiTect SYBR Green PCR Master Mix, miScript Universal Primer | 218073 |
| miScript Primer Assay (100) | miRNA-specific primer; available via GeneGlobe | Varies* |
| miScript Precursor Assay (100) | 10x miScript Precursor Assay (contains one miRNA-precursor–specific forward primer and one miRNA-precursor–specific reverse primer) | Varies* |
| Pathway-Focused miScript miRNA PCR Array | Array of assays for a pathway, disease, or gene family for human, mouse, rat, dog, or rhesus macaque miRNAs; available in 96-well, 384-well, or Rotor-Disc® 100 format | Varies |
| miRNome miScript miRNA PCR Array | Array of assays for the complete human, mouse, rat, dog, or rhesus macaque miRNome; available in 96-well, 384-well, or Rotor-Disc 100 format | Varies |
| QuantiTect Primer Assay (200) | Lyophilized primer mix of forward and reverse primers for SYBR Green based real-time RT-PCR. | Varies* |
| QuantiTect SYBR Green PCR Kit (200)† | For 200 x 50 µl reactions: 3 x 1.7 ml 2x QuantiTect SYBR Green PCR Master Mix, 2 x 2 ml RNase-Free Water | 204143 |

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

* Visit www.qiagen.com/GeneGlobe to search for and order these products.
† Other kit sizes are available; see www.qiagen.com.

## Notes

**Limited License Agreement for miRNeasy 96 Kit**

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

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