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RNeasy® Plus Universal Handbook

RNeasy Plus Universal Mini Kit

For purification of total RNA from all types of tissue

Sample to Insight

QIAGEN

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Kit Contents

RNeasy Plus Universal Mini Kit	**Mini (50)**
Catalog no.	**73404**
Number of preps	**50**
RNeasy Mini Spin Columns (pink) (each in a 2 ml Collection Tube)	50
Collection Tubes (1.5 ml)	50
Collection Tubes (2 ml)	50
QIAzol® Lysis Reagent*	50 ml
gDNA Eliminator Solution	8 ml
Buffer RWT*† (concentrate)	15 ml
Buffer RPE‡ (concentrate)	11 ml
RNase-Free Water	10 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.

Note: QIAzol Lysis Reagent is delivered separately.

Storage

RNeasy Plus Universal Mini Kit should be stored dry at room temperature (15–25°C). All components are stable for at least 9 months under these conditions, if not otherwise stated on the label.

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

Intended Use

RNeasy Plus Universal Mini Kit is intended for molecular biology applications. This product is not intended for the diagnosis, treatment or prevention of a disease.

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

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

Safety Information

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

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

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

Quality Control

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

Introduction

The RNeasy Plus Universal Mini Kit is designed for lysis of all kinds of tissues and subsequent purification of high-quality total RNA. gDNA Eliminator Solution effectively removes genomic DNA contamination eliminating the need for DNase digestion. It also significantly improves separation of DNA into the interphase. The RNeasy Plus Universal protocol is easy to follow and, compared to phenol/chloroform treatment with a subsequent cleanup procedure, much faster and more efficient with better RNA purity and yield. QIAGEN also provides a wide range of other kits for purification of total RNA from different sample sources (visit **www.qiagen.com/RNA**).

Principle and procedure

The RNeasy Plus Universal Mini Kit integrates phenol/guanidine-based sample lysis and silica-membrane purification of total RNA. QIAzol Lysis Reagent, included in the kit, is a monophasic solution of phenol and guanidine thiocyanate, designed to facilitate lysis of all kinds of tissues and inhibit RNases. The high lysis efficiency of the reagent and the subsequent removal of contaminants by organic phase extraction enable use of larger amounts of tissue with RNeasy spin columns. The RNeasy Plus Universal Mini Kit can be used to purify up to 50 mg of tissue (or up to 100 mg of brain or adipose tissue) per RNeasy Mini spin column*

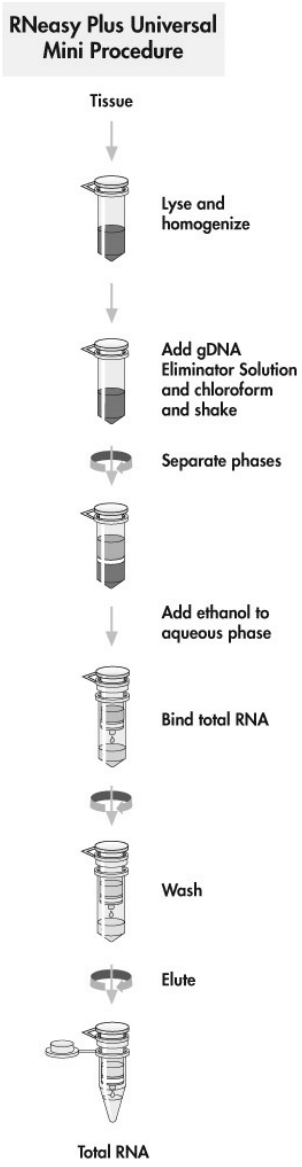
Tissue samples are homogenized in QIAzol Lysis Reagent. After addition of gDNA Eliminator Solution and chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase, while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase. The upper, aqueous phase is collected, and RNA is purified using RNeasy spin columns.

* To ensure optimal RNA yields, the binding capacity of the RNeasy spin column must not be exceeded. For details, see the protocol.

The upper, aqueous phase is mixed with ethanol to provide appropriate binding conditions and applied to an RNeasy Mini spin column. Total RNA binds to the spin column membrane, and phenol and other contaminants are efficiently washed away. High-quality RNA is then eluted in RNase-free water (see flowchart, page 8).

The standard protocol for RNeasy Plus Universal Mini Kit (page 16) allows purification of all RNA molecules longer than 200 nucleotides. The procedure provides an enrichment for mRNA, since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA and tRNAs, which together comprise 15–20% of total RNA) are selectively excluded. The size distribution of the purified RNA is comparable to that obtained by centrifugation through a CsCl gradient or cushion, where small RNAs do not sediment efficiently.

For purification of total RNA containing small RNAs, such as microRNA (miRNA) using the RNeasy Plus Universal Mini Kit, see Appendix C (page 35). QIAGEN also provides miRNeasy Kits for purification of miRNA, either in a total RNA fraction or in a fraction enriched in small RNAs. miRNeasy Kits are available in both spin-column and 96-well formats. For more details, visit **www.qiagen.com/product-categories/discovery-and-translational-research/dna-rna-purification/rna-purification/mirna/**.


RNeasy Plus Universal Mini Procedure
Tissue
Lyse and homogenize
Add gDNA Eliminator Solution and chloroform and shake
Separate phases
Add ethanol to aqueous phase
Bind total RNA
Wash
Elute
Total RNA

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.

- Chloroform
- Ethanol (70% and 96–100%)*
- Sterile, RNase-free pipet tips
- For stabilization of RNA in tissues (see page 12): RNAprotect® Tissue Reagent or Allprotect® Tissue Reagent (see ordering information, page 38) or liquid nitrogen and dry ice
- 1.5 ml or 2 ml microcentrifuge tubes
- Microcentrifuge(s) (with rotor for 2 ml tubes) for centrifugation at 4°C and at room temperature (15–25°C)
- Equipment for tissue disruption and homogenization (see page 13): we recommend the TissueRuptor® II, the TissueLyser LT or the TissueLyser II (see ordering information, page 38)

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

Important Notes

Determining the amount of starting material

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

- The type of tissue and its RNA content
- The volume of QIAzol Lysis Reagent required for efficient lysis
- The RNA binding capacity of the RNeasy Mini spin column

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

When processing samples containing low amounts of RNA, the maximum amount of starting material shown in Table 1 can be used. However, even though the RNA binding capacity of the RNeasy spin column is not reached, the maximum amount of starting material must not be exceeded. Otherwise, lysis will be incomplete and cellular debris may interfere with the binding of RNA to the RNeasy spin column membrane, resulting in lower RNA yield and purity.

More information on using the correct amount of starting material is given in the protocols. Table 2 shows expected RNA yields from various sources.

Table 1. RNeasy spin column specifications

Specification	RNeasy Mini spin column
Maximum binding capacity	100 µg RNA
Maximum loading volume	700 µl
RNA size distribution	RNA >200 nucleotides
Minimum elution volume	30 µl
Maximum amount of starting tissue	≤50 mg

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

Table 2. Typical yields of total RNA with RNeasy Plus Universal Mini Kit

Mouse/rat tissue (10 mg)	Yield of total RNA (µg)*
Adipose tissue	0.5–2.5
Brain	5–20
Heart	5–25
Intestine	10–60
Kidney	5–40
Liver	15–80
Lung	5–15
Muscle	5–35
Skin	2–5
Spleen	15–100

* Amounts can vary due to factors, such as species and developmental stage (especially with adipose tissues, large variations are possible due to developmental stage and location of the tissue). Since the RNeasy procedure enriches for mRNA and other RNA species >200 nucleotides, the total RNA yield does not include 5S rRNA, tRNA and other low-molecular-weight RNAs, which make up 15–20% of total cellular RNA.

Handling and storing starting material

RNA in harvested tissue is not protected until the sample is treated with RNAprotect Tissue Reagent, flash-frozen or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. Otherwise, unwanted changes in the gene expression profile will occur. It is therefore important that tissue samples are immediately frozen in liquid nitrogen and stored at –70°C or immediately immersed in RNAprotect Tissue Reagent at room temperature (15–25°C). An alternative to RNAprotect Tissue Reagent is Allprotect Tissue Reagent, which provides immediate stabilization of DNA, RNA and protein in tissue samples at room temperature.

Note: RNAprotect Tissue Reagent cannot be used to stabilize RNA in adipose tissue due to the high abundance of fat, but can be used to stabilize RNA in other fatty tissues, such as brain. Allprotect Tissue Reagent can stabilize adipose and brain tissue.

The procedures for tissue harvesting and RNA protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or weighing.

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. Incomplete disruption results in significantly reduced 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. Incomplete homogenization results in inefficient binding of RNA to the RNeasy spin column membrane and therefore significantly reduced RNA yields.

Disruption and homogenization of tissue samples can be carried out rapidly and efficiently using either the TissueRuptor II (for processing samples individually) or a TissueLyser system (for processing multiple samples simultaneously). Disruption and homogenization with TissueRuptor and TissueLyser systems generally results in higher RNA yields than with other methods.

Disruption and homogenization using the TissueRuptor II

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

Disruption and homogenization using TissueLyser systems

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. Two bead mills are available from QIAGEN: the TissueLyser LT for low- to medium-throughput disruption, and the TissueLyser II for medium- to high-throughput disruption.

The TissueLyser LT disrupts and homogenizes up to 12 samples at the same time. The instrument needs to be used in combination with the TissueLyser LT Adapter, which holds 12 x 2 ml microcentrifuge tubes containing stainless steel beads of 5 mm or 7 mm mean diameter. For guidelines on using the TissueLyser LT, refer to the *TissueLyser LT Handbook*.

The TissueLyser II disrupts and homogenizes up to 48 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 24, which holds 48 x 2 ml microcentrifuge tubes containing stainless steel beads of 5 mm mean diameter. For guidelines on using the TissueLyser II, refer to the *TissueLyser Handbook*. If using other bead mills for sample disruption and homogenization, refer to suppliers' guidelines.

Note: Tungsten carbide beads react with QIAzol Lysis Reagent and must not be used to disrupt and homogenize tissues.

The TissueLyser II can also disrupt and homogenize up to 192 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 96, which holds 192 x 1.2 ml microtubes containing stainless steel beads of 5 mm mean diameter. In this case, we recommend using the RNeasy 96 Universal Tissue Kit, which provides high-throughput RNA purification from all types of tissue in 96-well format. For ordering information, see page 38.

Automated purification of RNA on QIAcube Instruments

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

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

QIAcube Connect.

Protocol: Purification of Total RNA using the RNeasy Plus Universal Mini Kit

Determining the correct amount of starting material

It is essential to use the correct amount of tissue to obtain optimal RNA yield and purity. With the RNeasy Plus Universal Mini Kit, a maximum of 50 mg tissue can generally be processed. Using this amount, the RNA binding capacity of the RNeasy Mini spin column and the lysing capacity of QIAzol Lysis Reagent will not be exceeded. For brain or adipose tissue, a maximum of 100 mg tissue can generally be used. For tissues with high RNA content, such as liver, spleen and thymus, we recommend using no more than 30 mg tissue to ensure optimal RNA yields and to avoid exceeding the binding capacity of the RNA spin column. Average RNA yields from various tissues are given in Table 2 (page 11).

If there is no information about the nature of your starting material, we recommend starting with no more than 30 mg tissue. Depending on RNA yield and purity, it may be possible to use up to 100 mg tissue in subsequent preparations.

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

Weighing tissue is the most accurate way to quantify the amount of starting material. As a guide, a 4 mm cube (64 mm^3) of most animal tissues weighs 70–85 mg.

Important points before starting

- If using RNeasy Plus Universal Mini Kits for the first time, read "Important Notes" (page 10).
- If working with RNA for the first time, read Appendix A (page 28).
- If using a TissueRuptor or TissueLyser system, ensure that you are familiar with operating it by referring to the supplied user manual (operating instructions) and handbook.
- To freeze tissue for long-term storage (several months), flash-freeze in liquid nitrogen and immediately transfer to –70°C. Do not allow tissues to thaw during weighing or handling prior to disruption in QIAzol Lysis Reagent. Homogenized tissue lysates from step 3 can also be stored at –70°C for several months. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 4. Avoid prolonged incubation, which may compromise RNA integrity.
- Generally, DNase digestion is not required since integrated QIAzol, gDNA Eliminator Solution and RNeasy technologies efficiently remove most of the genomic DNA contamination without DNase treatment. For real-time two-step RT-PCR applications, further DNA removal can be achieved using the QuantiNova® Reverse Transcription Kit, which provides cDNA synthesis with integrated removal of genomic DNA contamination (see ordering information, page 38).
- 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.
- Except for phase separation (step 8), all protocol and centrifugation steps should be performed at room temperature (15–25°C). During the procedure, work quickly.

Things to do before starting

- 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.

Procedure

1. If using a TissueLyser system, add one stainless steel bead (5 mm mean diameter) per 2 ml microcentrifuge tube (not supplied). If working with tissues that are not stabilized in RNAprotect- or Allprotect-Reagent, place the tubes on dry ice.

   Note: When disrupting tough or very tough samples with the TissueLyser LT, we recommend using one or two 7 mm stainless steel beads.

2. Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue. Do not use more than 50 mg tissue or more than 100 mg brain or adipose tissue. Proceed immediately to step 3.

   Weighing tissue is the most accurate way to determine the amount.

   If the tissue sample was stored in RNAprotect Tissue Reagent or Allprotect Reagent, remove it from the reagent using forceps and be sure to remove any excess reagent or crystals that may have formed.

   RNA in harvested tissues is not protected until the tissues are treated with RNAprotect Tissue Reagent or Allprotect Reagent, flash-frozen or disrupted and homogenized in step 3. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

3. Disrupt the tissue and homogenize the lysate using the TissueRuptor II (follow step 3a), TissueLyser LT (follow step 3b) or TissueLyser II (follow step 3c).

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

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

3a. Disruption and homogenization using the TissueRuptor II:

- Place the tissue in a suitably sized vessel containing 900 µl QIAzol Lysis Reagent.

Note: Use a suitably sized vessel with sufficient extra headspace to accommodate foaming, which may occur during homogenization.

Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.

- **Place the tip of** the **disposable probe into the vessel and operate the TissueRuptor II at full speed until the lysate is uniformly homogeneous (usually 20–40 s). Proceed to step 4.**

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

Foaming may occur during homogenization, especially of brain tissue. If this occurs, let the homogenate stand at room temperature (15–25°C) for 2–3 min until the foam subsides before continuing with the procedure.

3b. Disruption and homogenization using the TissueLyser LT:

- **Keep the tubes prepared in step 1 on dry ice for at least 15 min (however, keep the insert of the TissueLyser LT Adapter at room temperature). Then place the tissues in the tubes, and keep the tubes on dry ice for another 15 min.**

If working with RNAprotect- or Allprotect-stabilized tissues, it is not necessary to place the tubes on dry ice.

- **Place the tubes in the insert of the TissueLyser LT Adapter, and incubate at room temperature for 2 min. Then immediately add 900 µl QIAzol Lysis Reagent per tube.**

Do not incubate for longer than 2 min, otherwise frozen tissues will thaw, resulting in potential RNA degradation.

- **Place the tubes in the TissueLyser LT Adapter.**
- **Operate the TissueLyser LT for 2–5 min at 50 Hz.**

The time depends on the tissue being processed and can be extended until the tissue is completely homogenized.

- **Carefully pipet the lysates into new microcentrifuge tubes (not supplied). Proceed to step 4.**

Do not reuse the stainless-steel beads.

3c. Disruption and homogenization using the TissueLyser II:

- **Place the tissues in the tubes prepared in step 1.**
- **If the tubes were stored on dry ice, place them at room temperature. Then immediately add 900 µl QIAzol Lysis Reagent per tube.**
- **Place the tubes in the TissueLyser Adapter Set 2 x 24.**
- **Operate the TissueLyser II for 2 min at 20 Hz.**

The time depends on the tissue being processed and can be extended until the tissue is completely homogenized.

- **Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser II for another 2 min at 20 Hz.**

Rearranging the tubes allows even homogenization.

- **Carefully pipet the lysates into new microcentrifuge tubes (not supplied). Proceed to step 4.**

Do not reuse the stainless-steel beads.

4. Place the tube containing the homogenate on the benchtop at room temperature for 5 min.

This step promotes dissociation of nucleoprotein complexes.

5. Add 100 µl gDNA Eliminator Solution. Securely cap the tube containing the homogenate, and shake it vigorously for 15 s.

   Addition of gDNA Eliminator Solution will effectively reduce genomic DNA contamination of the aqueous phase, making further treatment with DNase unnecessary.

6. Add 180 µl chloroform. Securely cap the tube containing the homogenate, and shake it vigorously for 15 s.

   Thorough mixing is important for subsequent phase separation.

7. Place the tube containing the homogenate on the benchtop at room temperature for 2–3 min.

8. Centrifuge at 12,000 x *g* for 15 min at 4°C. After centrifugation, heat the centrifuge to room temperature if the same centrifuge will be used in the later steps of this procedure.

   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 600 µl.

9. Transfer the upper, aqueous phase (usually 600 µl) to a new microcentrifuge tube (not supplied).

10. Add 1 volume (usually 600 µl) of 70% ethanol, and mix thoroughly by pipetting up and down. Do not centrifuge. Proceed immediately to step 11.

    Note: The volume of lysate may be less than 600 µl due to loss during homogenization and centrifugation.

    Precipitates may be visible after addition of ethanol. Resuspend precipitates completely by vigorous shaking, and proceed immediately to step 11.

11. Transfer up to 700 µl of the sample to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) at room temperature. Discard the flow-through.*

    Reuse the collection tube in step 12.

12. Repeat step 11 using the remainder of the sample. Discard the flow-through.*

    Reuse the collection tube in step 13.

13. Add 700 µl Buffer RWT to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the membrane. Discard the flow-through.*

    Reuse the collection tube in step 14.

    After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.*

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

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

    Reuse the collection tube in step 15.

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

* Flow-through contains QIAzol Lysis Reagent or Buffer RWT and is therefore not compatible with bleach. See page 6 for safety information.

15. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the membrane.

    The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

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

16. **Optional**: Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min.

    Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 15.

17. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently. To elute the RNA, centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm).

18. Repeat step 17 using another volume of RNase-free water, or using the eluate from step 17 (if high RNA concentration is required). Reuse the collection tube from step 17.

    If using the eluate from step 17, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

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 (step 6), 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 in steps 7 and 8.
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 RNeasy spin column	
a) Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting material" (page 13) for details on disruption and homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see page 10 and protocol, page 16) and/or increase the homogenization time.
b) Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see page 10 and protocol, page 16).

		Comments and suggestions
c)	Centrifugation temperature too low	Except for phase separation (step 8), all centrifugation steps should be performed at 15–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the RNeasy spin column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol-containing lysate to 37°C before transferring to the RNeasy spin column.
Low RNA yield		
a)	Insufficient disruption and homogenization	See "Disrupting and homogenizing starting material" (page 13) for details on disruption and homogenization methods.
b)	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see page 10 and protocol, page 16).
c)	RNA still bound to RNeasy spin column membrane	Repeat RNA elution, but incubate the RNeasy spin column on the benchtop for 10 min with RNase-free water before centrifuging.
d)	Centrifugation temperature too low	Except for phase separation (step 8), all centrifugation steps should be performed at 15–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the RNeasy spin column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol-containing lysate to 37°C before transferring to the RNeasy spin column.
Low or no recovery of RNA		
	RNase-free water incorrectly dispensed	Add RNase-free water to the center of the RNeasy spin column membrane to ensure that the membrane is completely covered.
Low A_{260}/A_{280} value		
a)	Not enough QIAzol Lysis Reagent used for homogenization	In subsequent preparations, 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 protocol (step 4). 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 B, page 31).
RNA degraded		
a)	Inappropriate handling of starting material	For frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the RNeasy procedure quickly, especially the first few steps. See Appendix A (page 28) and "Handling and storing starting material" (page 12).
b)	RNase contamination	Although all RNeasy buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the RNeasy procedure or later handling. See Appendix A (page 28) for general remarks on handling RNA. Do not put RNA samples into a vacuum dryer that has been used in DNA preparation where RNases may have been used.
DNA contamination in downstream experiments		
a)	Phase separation performed at too high a temperature	The phase separation (step 8) 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.
b)	Interphase contamination of aqueous phase	Contamination of the aqueous phase with the interphase results in an increased DNA content in the RNA eluate. Make sure to transfer the aqueous phase without interphase contamination.
c)	Not enough QIAzol Lysis Reagent used for homogenization	In subsequent preparations, reduce the amount of starting material and/or increase the volume of QIAzol Lysis Reagent and the homogenization time.

		Comments and suggestions
d)	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.
e)	No gDNA Eliminator Solution added before phase separation	In subsequent preparations, add the appropriate amount of gDNA Eliminator Solution.
f)	Lysate mixed with chloroform by vortexing	Mix lysate and chloroform by shaking vigorously. Do not vortex the tubes.
RNA does not perform well in downstream experiments		
a)	Salt carryover during elution	Ensure that Buffer RPE is at 20–30°C.
b)	Ethanol carryover	During the second wash with Buffer RPE, be sure to dry the RNeasy spin column membrane by centrifuging at ≥8000 x *g* (≥10,000 rpm) for 2 min at 15–25°C. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur. To eliminate any chance of possible ethanol carryover with the RNeasy Plus Universal Mini Kit, place the RNeasy Mini spin column in a new 2 ml collection tube and perform the optional 1-min centrifugation step as described in step 16 of the protocol.

Appendix A: 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 destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. 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 general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA,* followed by RNase-free water (see "Solutions", page 29), 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, then rinse with ethanol (if the tanks are ethanol resistant) and allow to dry.

* 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.

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 safety data sheets (SDSs), available from the product supplier. 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 at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with diethyl pyrocarbonate (DEPC)*, 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 has been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

* 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.

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

Appendix B: Storage, Quantification and Determination of Quality of RNA

Storage of RNA

Purified RNA may be stored at –70°C to –15°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 quantified using quantitative RT-PCR or fluorometric quantification.

Spectrophotometric quantification of RNA

Using the QIAxpert UV/VIS Spectrophotometer for microvolume analysis

To determine the concentration of your RNA sample purified with RNeasy QIAGEN kit, use the corresponding RNeasy App on the QIAxpert. For more information, see the QIAxpert product page (**www.qiagen.com/qiaxpert-system**).

Using a standard spectrophotometer

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 → 4 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 32), 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 29). 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 assessment of RNA purity will be performed routinely, when using the QIAxpert with the corresponding RNeasy App. See the QIAxpert user manual for more information (**www.qiagen.com/qiaxpert-system/user manual**)

* 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 standard photometric measurements, 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, such as protein, that absorb in the UV spectrum. 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 when using pure water. 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 "Spectrophotometric quantification of RNA", page 31).

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. RNeasy Kits will, however, remove the vast majority of cellular DNA. gDNA Eliminator Solution helps to further reduce genomic DNA contamination; however, trace amounts of genomic DNA 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 real-time RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® 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 sequences (without detection of genomic DNA) where possible (see **www.qiagen.com/GeneGlobe**). For real-time RT-PCR assays where amplification of genomic

* 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.

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 (see ordering information, page 38).

Integrity of RNA

The integrity and size distribution of total RNA purified with RNeasy Plus Universal Mini Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxcel system or Agilent® 2100 Bioanalyzer. Ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S rRNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the sample suffered major degradation either before or during RNA purification. As a useful measure of RNA integrity, the QIAxcel® Advanced system and the Agilent 2100 Bioanalyzer provide an RNA integrity score (RIS) and an RNA integrity number (RIN), respectively. Ideally, the value 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.

Appendix C: Purification of Total RNA Containing miRNA using the RNeasy Plus Universal Mini Kit

The RNeasy Plus Universal Mini Kit can also be used to purify total RNA that contains small RNAs, such as miRNA.

Procedure

Follow the protocol on pages 16–21, up to and including step 9, and then follow steps 1–9 below.

1. Add 1.5 volumes (usually 900 µl) of 100% ethanol, and mix thoroughly by pipetting up and down. Do not centrifuge. Proceed immediately to step 2.

   Note: The volume of lysate may be less than 600 µl due to loss during homogenization and centrifugation.

   Precipitates may be visible after addition of ethanol. Resuspend precipitates completely by vigorous shaking, and proceed immediately to step 2.

2. Transfer up to 700 µl of the sample to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) at room temperature (15–25°C). Discard the flow-through.*

   Reuse the collection tube in step 3.

3. Repeat step 2 using the remainder of the sample. Discard the flow-through.*

   Reuse the collection tube in step 4.

* Flow-through contains QIAzol Lysis Reagent or Buffer RWT and is therefore not compatible with bleach. See page 6 for safety information.

4. Add 700 µl Buffer RWT to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the membrane. Discard the flow-through.*

   Reuse the collection tube in step 5.

   After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.*

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

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

   Reuse the collection tube in step 6.

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

6. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the membrane.

   The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

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

7. **Optional**: Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min.

   Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 6.

8. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently. To elute the RNA, centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm).
9. Repeat step 8 using another volume of RNase-free water, or using the eluate from step 8 (if high RNA concentration is required). Reuse the collection tube from step 8.

   If using the eluate from step 8, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Ordering Information

Product	Contents	Cat. no.
RNeasy Plus Universal Mini Kit (50)	For 50 RNA minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Solution, Collection Tubes, RNase-Free Water and Buffers	73404
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395
Related products		
Allprotect Tissue Reagent (100 ml)	100 ml Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
RNAprotect Tissue Reagent (50 ml)	50 ml RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104
RNAprotect Tissue Reagent (250 ml)	250 ml RNAprotect Tissue Reagent for stabilization of RNA in 125 x 200 mg tissue samples	76106
RNAprotect Tissue Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 50 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNAprotect Tissue Reagent each	76154

Product	Contents	Cat. no.
RNAprotect Tissue Tubes (20 x 5 ml)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNAprotect Tissue Reagent each	76163
QIAzol Lysis Reagent (200 ml)	200 ml QIAzol Lysis Reagent	79306
RNeasy 96 Universal Tissue Kit (4)	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Collection Microtubes, Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	74881
TissueRuptor II	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	Varies
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor II	990890
TissueLyser LT	Compact bead mill, 100–240 V AC, 50–60 Hz; requires the TissueLyser LT Adapter, 12-Tube (available separately)	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
Sample Tubes RB (2 ml)	1000 safe-lock microcentrifuge tubes (2 ml) for use with the TissueLyser LT	990381
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

Product	Contents	Cat. no.
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
Collection Microtubes (racked)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
Collection Microtube Caps (120 x 8)	Nonsterile polypropylene caps for collection microtubes (1.2 ml), 960 in strips of 8	19566
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with TissueLyser systems	69989
Stainless Steel Beads, 7 mm (200)	Stainless Steel Beads, suitable for use with TissueLyser systems	69990
RNase-Free DNase Set (50)	1500 Kunitz units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA minipreps	79254
Collection Tubes (2 ml)	1000 x 2 ml Collection Tubes	19201
QuantiNova Rev. Transcription Kit (10)	For 10 x 20 µl reactions: 20 µl 8x gDNA Removal Mix, 10 µl Reverse Transcription Enzyme, 40 µl Reverse Transcription Mix (containing RT primers), 20 µl Internal Control RNA, 1.9 ml RNase-Free Water	205410

Product	Contents	Cat. no.
QuantiNova Rev. Transcription Kit (50)	For 50 x 20 µl reactions: 100 µl 8x gDNA Removal Mix, 50 µl Reverse Transcription Enzyme, 200 µl Reverse Transcription Mix (containing RT primers), 100 µl Internal Control RNA, 1.9 ml RNase-Free Water	205411

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

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.

Document Revision History

Date	Changes
April 2021	Removed mention of discontinued product. Updated text, ordering information and intended use for QIAcube Connect. Updated branding of RNA protection products.

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