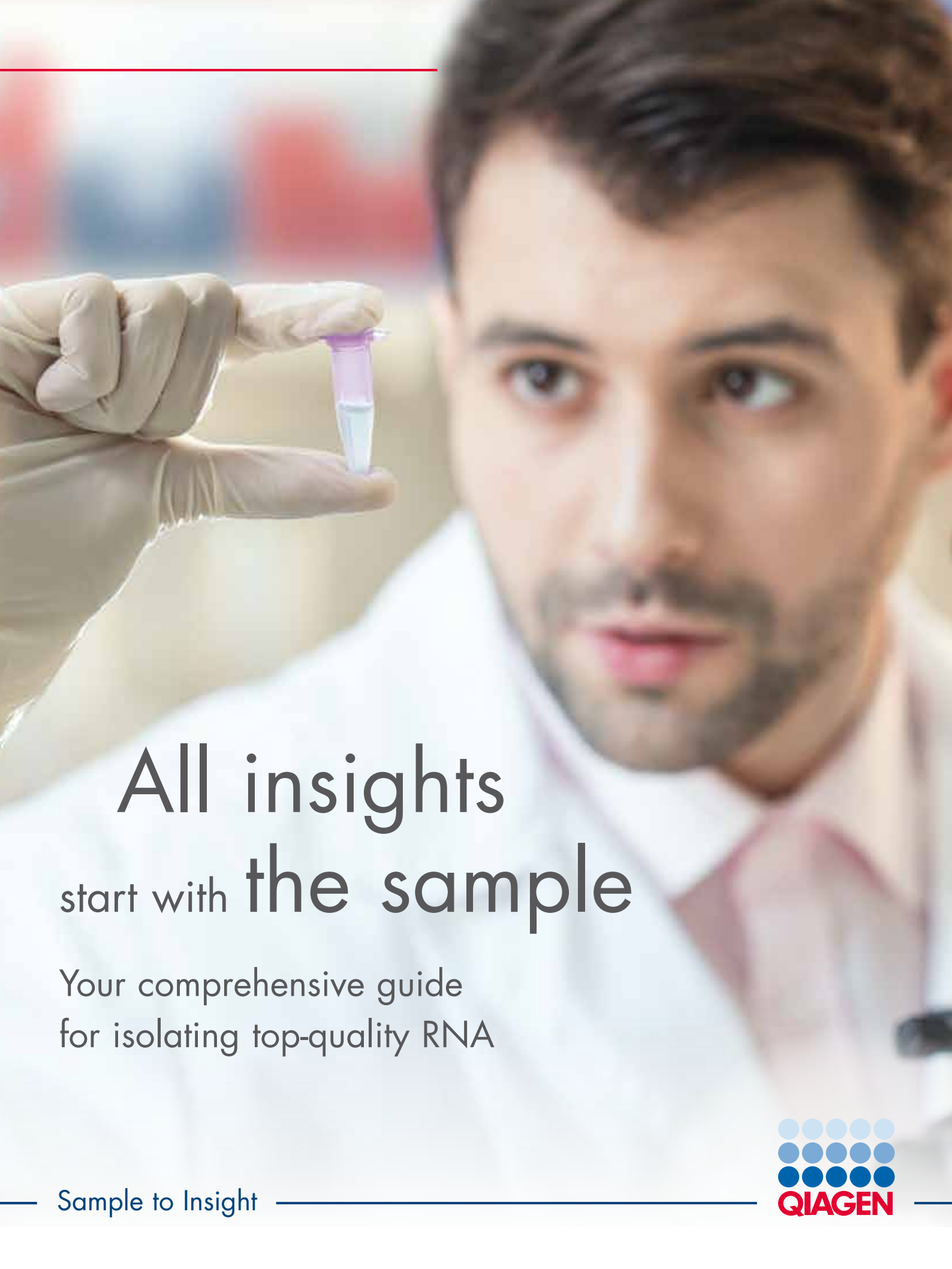


All insights start with the sample

Your comprehensive guide for isolating top-quality RNA

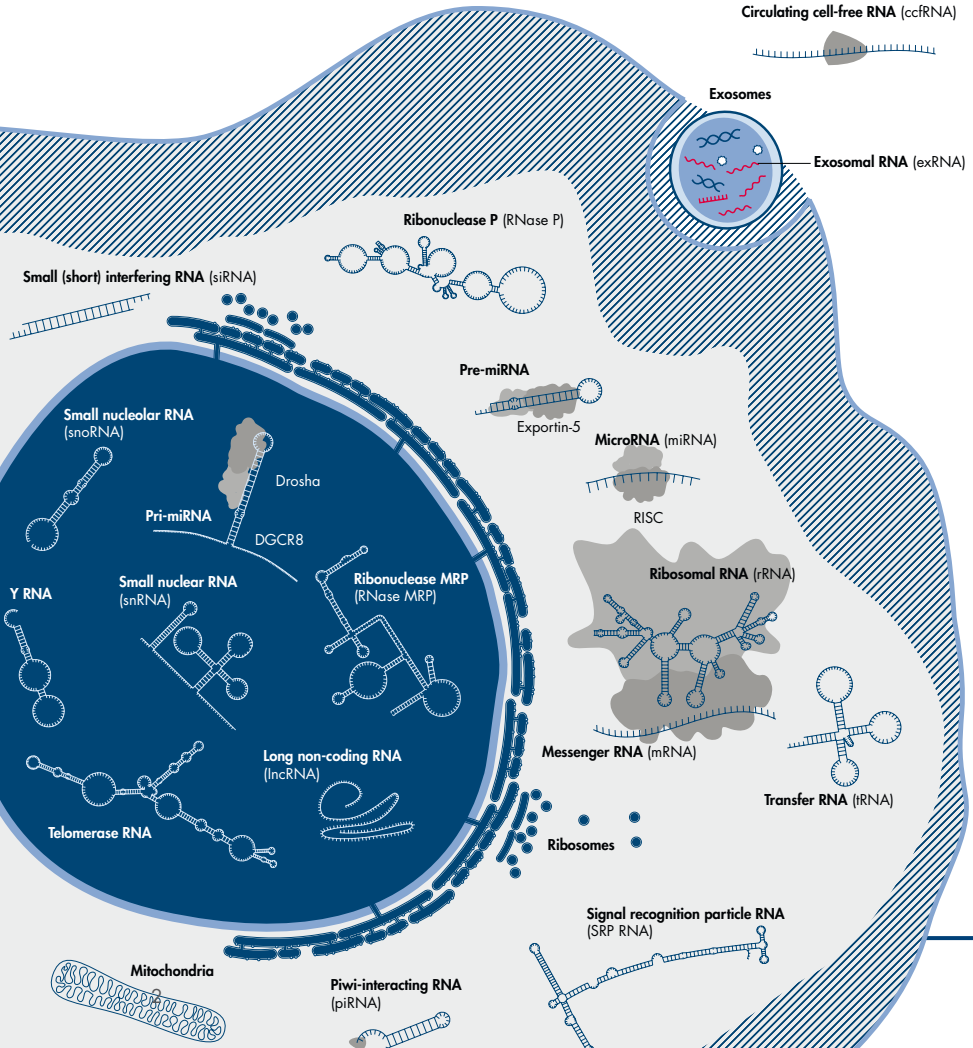
Sample to Insight

QIAGEN

Explore the RNA universe!

RNAs have a number of different functions in a cell. Messenger RNAs (mRNA) are generated in the nucleus and serve as templates for synthesis of proteins in the cytosol. Protein synthesis is carried out by ribosomes, which consist of ribosomal RNA (rRNA) and proteins. Amino acids for protein synthesis are delivered to the ribosome on transfer RNA (tRNA) molecules. Other non-coding RNAs, such as long noncoding RNA (lncRNA), small nucleolar RNA (snoRNA), microRNA (miRNA), short interfering RNA (siRNA) or piwi-interacting RNA (piRNA) are involved in the regulation of gene expression. RNAs are also part of riboproteins involved in RNA processing.

A typical rapidly growing mammalian cell culture contains 10–30 pg total RNA per cell, while a fully differentiated primary cell will contain far less, closer to ~1 pg per cell. The majority of RNA molecules are tRNAs and rRNAs. mRNA accounts for only 3–7% of the total cellular RNA, although the actual amount depends on the cell type and physiological state (see Figure 1). Analysis of RNA can provide a good reflection of an organism's gene expression profile under a given set of conditions. Reliable results in RNA profiling methods such as real-time RT-PCR or whole transcriptome sequencing depend strongly on the quality of the RNA sample used and how it is processed. To ensure success in every step of your RNA experiments, QIAGEN provides a comprehensive range of technologies, including stabilization and disruption technologies.



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www.qiagen.com/RNA-Poster

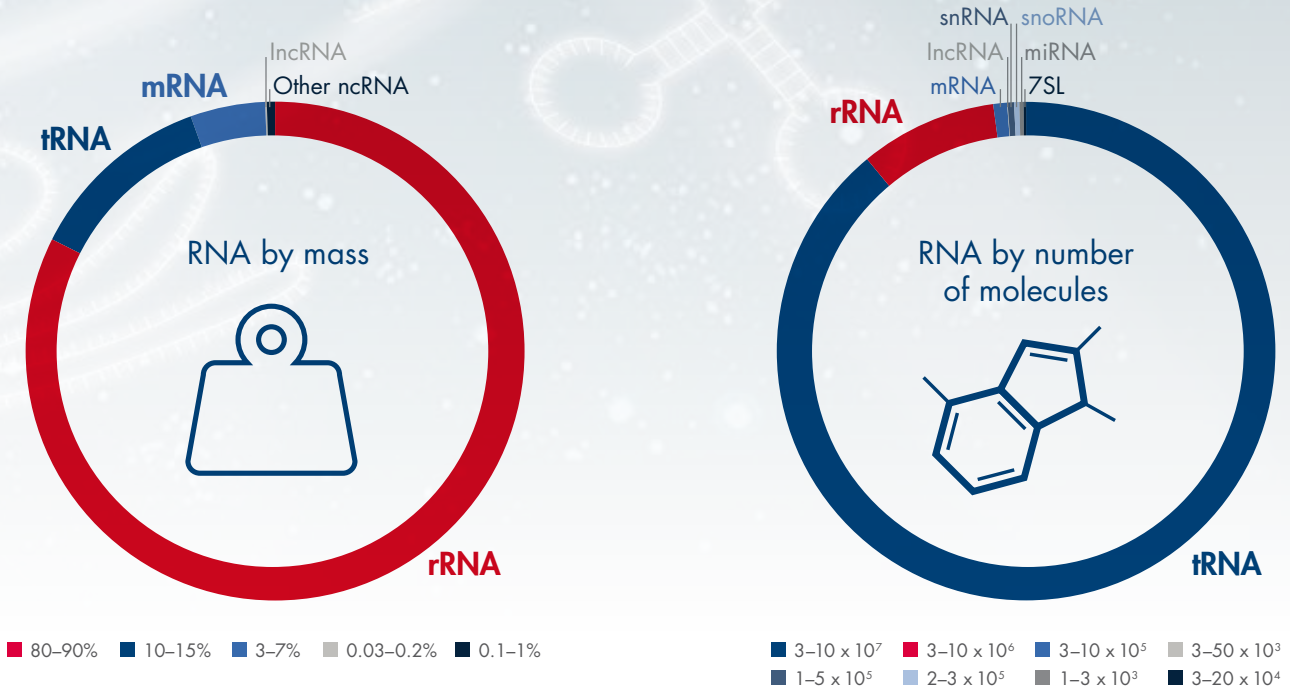

Figure 1. RNA distribution in a typical mammalian cell. (Palazzo, A.F. and Lee, E.S. (2015) Non-coding RNA: what is functional and what is junk? Front. Genet. 6:2)

Achieving reproducible results

The concern around scientific research reproducibility has been growing for some time now. High-quality science is critical, as reproducible and reliable studies lay the foundation for future scientific endeavors to build upon. However, many scientists are unable to reproduce the results of their peers as well as their own results. This irreproducibility crisis wastes valuable time and resources and can damage scientific credibility.

Fortunately, you can easily increase the reliability of your RNA experiments by standardizing sample purifications and implementing quality control (QC) checks at key workflow steps. High-quality sample stabilization and RNA purification chemistries protect your samples from degradation and remove contaminants that interfere with downstream assays. Sample QC can detect small variations in sample quality and inform you if samples have been compromised during handling. Taking preventive or corrective measures early and processing only the highest quality samples increase experimental reproducibility and give you confidence in the quality of your results.

Sample collection and stabilization

When a biological sample is harvested, the RNA expression profile in the cells is altered. Sample collection and handling can cause *ex vivo* gene induction or down-regulation, leading to a respective increase or decrease in RNA levels (Figure 2). RNA degradation by RNases and initiation of cell death programs can also contribute to changes in cellular RNA transcript levels (Figure 2). Thus immediate stabilization of cellular RNA to preserve mRNA levels is critical for accurate gene expression analysis and allows capturing a snapshot of the true gene expression profile at the time of sample collection.

Traditionally, samples harvested for RNA analysis are immediately frozen in liquid nitrogen and stored at –80°C until processing. However, liquid nitrogen is hazardous and is not always available at the point of sample collection. Today, a variety of RNA collection and stabilization solutions are available that allow convenient, easy and safe sample handling and preservation of RNA at room temperature (see product selection guide, Table 6, page 17). For integrated sample collection, stabilization and purification of human blood, bone marrow or tissue samples, PAXgene® Systems are available, consisting of collection devices and sample purification kits. To find out more, visit **www.PreAnalytiX.com**.

QIAGEN RNA stabilization technologies enable:

- Immediate RNA stabilization: preserves RNA profile and systematically depletes sample contaminants for reliable gene expression analysis
- Safe sample thawing, easy handling and preparation at room temperature: no need for hazardous liquid nitrogen or dry ice to freeze samples
- Convenient shipping of samples: no impact of temperature fluctuations during transport and storage on intracellular RNA profile and RNA integrity
- Archiving of samples: long-term storage without RNA degradation

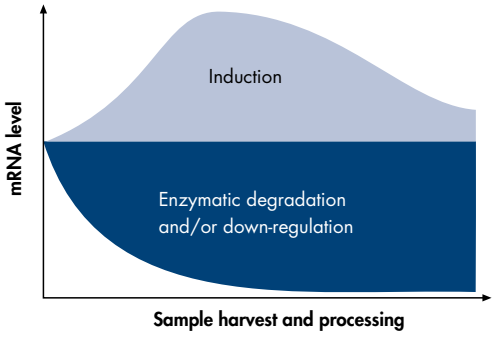

Figure 2. Illustration of changes in mRNA levels following sample harvesting.

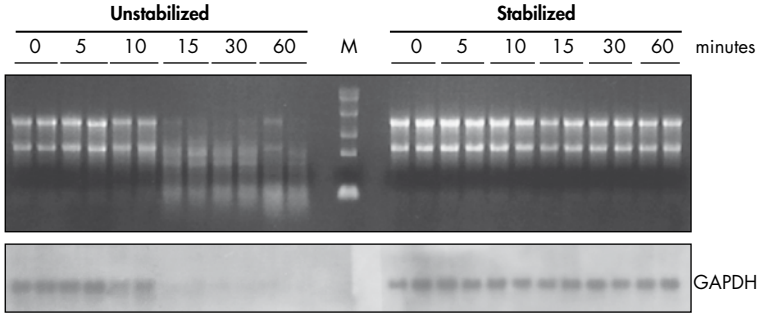

Figure 3. Purification of nondegraded RNA from stabilized samples. Rat kidneys were either immediately stabilized in RNA*later*® Reagent (Stabilized) or left unstabilized (Unstabilized). After 0–60 minutes, RNA was purified from stabilized and unstabilized samples and analyzed.

Sample disruption and homogenization

Effective sample disruption and homogenization is an absolute requirement for all RNA purification procedures. Incomplete disruption and homogenization reduces RNA yields and can lead to bias in RNA profiling. Sample disruption does make the RNA accessible to RNases, so it is crucial to perform this step under conditions that denature RNases.

QIAGEN provides a range of disruption and homogenization technologies, from dedicated lysis buffers and QIAshredder spin columns for fast and simple lysis and homogenization of cell culture samples (Table 1) to TissueRuptor® II and TissueLyser II and LT systems for mechanical disruption and homogenization of tougher samples at a range of throughputs (Table 2).

- Disruption releases the sample's RNA contained by breaking tissue structures, cell walls and membranes
- Homogenization reduces sample viscosity after disruption to facilitate subsequent RNA purification

Table 1: Disruption and homogenization methods for various starting materials.

Starting material	Disruption method	Homogenization method
Cells	Addition of lysis buffer and enzymes	Vortex, QIAshredder
Tissues	Rotor-stator homogenizer	Rotor-stator homogenizer
Tissues	Bead mill	Bead mill
Bacteria/Yeast	Enzymatic (lysozyme) digestion followed by addition of lysis buffer	Vortex
Bacteria/Yeast	Bead mill	Bead mill
Plants and filamentous fungi	Mortar and pestle	QIAshredder column

Table 2: TissueRuptor and TissueLyser systems deliver fast and effective disruption and homogenization and replace tedious and time-consuming methods such as manual disruption using a mortar and pestle.

TissueRuptor II	TissueLyser LT	TissueLyser II
1 sample per run	Up to 12 samples per run	Up to 48 or 192 samples per run
Hand-held rotor-stator homogenizer for simultaneous disruption and homogenization	Compact, cost-effective bead mill for disruption and homogenization	Medium to high-throughput bead mill for disruption and homogenization
Human/animal tissues Plant tissues	Human/animal tissues Plant tissues Bacteria/Yeast	
• Minimal risk of cross-contamination • Efficient sample disruption • Fast exchange of disposable probes eliminates need to clean the probe	• Convenient and time-saving processing of multiple samples • High-speed shaking of grinding beads in plastic tubes efficiently disrupts a broad range of sample types	

Disruption and homogenization using bead mills

In disruption using a bead mill, the sample is agitated at high speed in the presence of beads. Disruption and simultaneous homogenization occur by the hydrodynamic shearing and crushing action of the beads as they collide with the cells.

Disruption efficiency is influenced by the following factors:

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

Recommended beads for different sample types:

- **Bacteria:** 0.1 mm glass beads
- **Yeast and unicellular animal cells:** 0.5 mm glass beads
- **Animal and plant tissues:** 3–7 mm stainless steel beads

Stainless steel beads and other large beads disrupt macroscopic tissue very efficiently but are ineffective for small bacterial and animal cells. To ensure effective lysis for bacteria, we recommend using glass beads. Tungsten carbide beads can be used for lysis of fresh, frozen or lyophilized plant samples precooled on dry ice without lysis buffer.

Disruption and homogenization using rotor–stator adapters

Rotor–stator homogenizers thoroughly disrupt and homogenize animal tissues in lysis buffer or cell lysates in 5–90 seconds, depending on the toughness of the sample. The rotor turns at a very high speed to disrupt the sample and homogenizes it through a combination of turbulence and mechanical shearing.

The following tips will help you avoid sample foaming with rotor–stator homogenizers:

- Use a properly sized vessel
- Keep the tip of the homogenizer submerged
- Hold the immersed tip to one side of the tube

RNA isolation

When isolating RNA, it is important to use a method that maintains RNA integrity and removes contaminants. RNA degradation makes reliable gene expression analysis impossible, and the presence of contaminants in the purified RNA can inhibit enzymes in downstream applications such as real-time RT-PCR and next-generation sequencing (NGS). Sample compositions vary, requiring special considerations for RNA isolation from different sample sources. Our comprehensive portfolio of RNA purification kits helps you overcome these challenges through the combination of dedicated protocols, specialized lysis and wash buffers and proven silica-membrane spin-column technology. Use our product selection guides in Tables 6–11 on pages 17–20 to find the right RNA isolation kit for your sample type.

Spin-column technology

QIAGEN spin-column protocols consist of these four simple steps (Figure 4):

- Lyse – efficient lysis of the sample releases RNA content and inactivates RNases
- Bind – optimal conditions bind the desired RNA type to the silica membrane
- Wash – washing the silica membrane removes contaminants and cellular material
- Elute – RNA is released from the silica membrane and collected for downstream assay

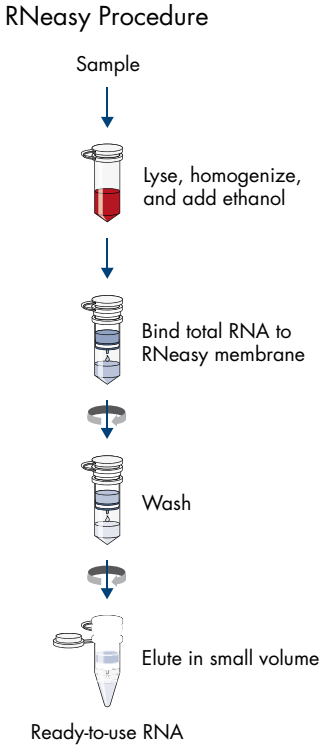

Figure 4. Typical RNA isolation procedure using QIAGEN spin-column technology.

Benefits of QIAGEN RNA isolation kits

- **Highly efficient removal of contaminants:** High-purity RNA suitable for all downstream applications
- **Dedicated products:** Optimized purification from a broad variety of sample types
- **Maximal yield:** Unbiased snapshot of RNA composition

Table 3: Sample and binding capacities of different spin column sizes.

Spin column size	Sample capacity	Binding capacity
Micro	<5x10^5 cells; <5 mg tissue	45 µg RNA
Mini	5x10^5 – 1x10^7 cells; 0.5–30 mg tissue	100 µg RNA
Midi	5x10^6 – 1x10^8 cells; 20–250 mg tissue	1 mg RNA
Maxi	5x10^7 – 5x10^8 cells; 150 mg – 1 g tissue	6 mg RNA

RNeasy® Kits preferentially isolate RNA larger than 200 nucleotides, enriching for mRNA and other long RNA transcripts, while miRNeasy® kits enable isolation of total RNA, including small RNA from approximately 18 nucleotides upwards. With miRNeasy kits, separate miRNA- and mRNA-enriched fractions can be isolated for all sample types, except samples containing severely degraded RNA, such as FFPE samples. Isolation of total RNA, including miRNA, allows direct comparison of miRNA expression levels with those of target mRNAs, housekeeping reference genes or any other mRNA of interest (Figure 5).

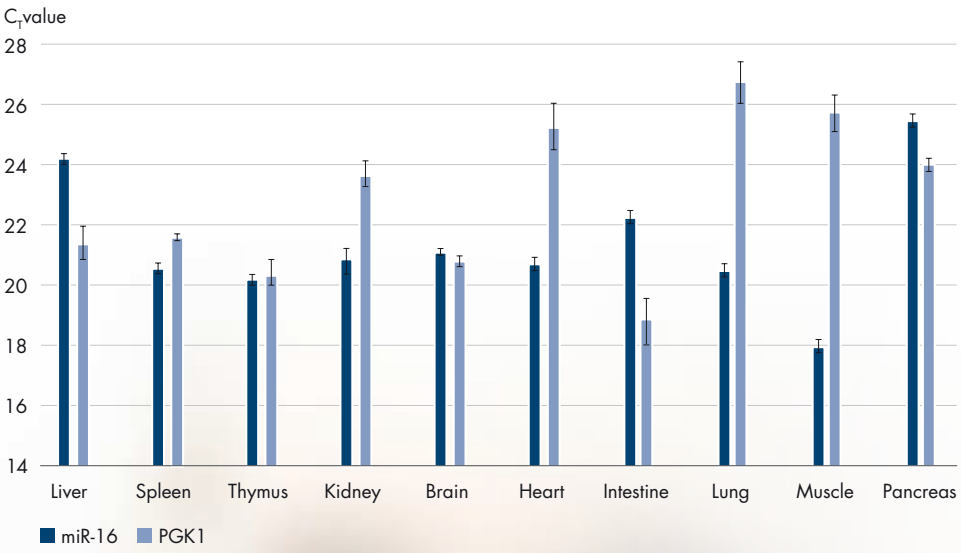

Figure 5. Efficient copurification of miRNA and RNA from a wide range of tissues. Total RNA including miRNA was purified from 25 mg of RNA*later*-stabilized rat tissues using the miRNeasy 96 Kit. miR-16 and PGK1 were quantified with qRT-PCR assays.

Did you know?

QIAGEN invented the spin-column technology more than 30 years ago and revolutionized how nucleic acids are purified from any biological sample!

High-purity RNA from FFPE tissue sections

Crosslinking and fragmentation can make purification of nucleic acids from FFPE sections challenging. But the RNeasy and miRNeasy FFPE Kits provide special lysis and incubation conditions including Proteinase K to efficiently release all RNA from tissue sections. Plus, optimized buffers and incubation conditions reverse crosslinking and formaldehyde modification of RNA, while avoiding further RNA degradation (Figure 6). To remove remaining small DNA fragments, RNA is treated with DNase in solution, ensuring optimal digestion efficiency. Total RNA can then be used directly in any downstream application.

The PAXgene Tissue System allows simultaneous and crosslinking-free stabilization of molecular content and preservation of histomorphology. Stabilized samples can be embedded in paraffin for histological studies, and RNA and miRNA can be isolated from the stabilized samples before or after embedding in paraffin.

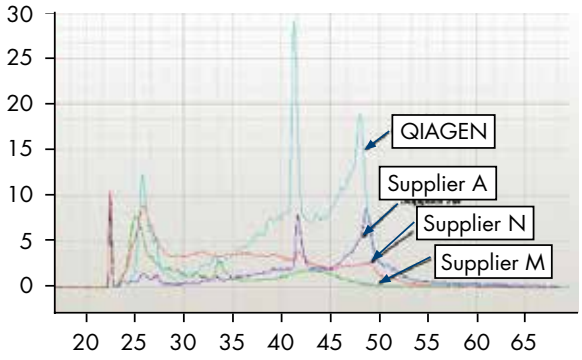

Figure 6. Recovery of all usable RNA. RNA purified from 6-month old FFPE rat liver using the RNeasy FFPE Kit or a kit from other suppliers was analyzed on the Agilent® Bioanalyzer.

Cell-free RNA purification from serum or plasma samples

When released by apoptotic and necrotic cells, most RNA molecules are degraded by RNases, which are abundant in the plasma and serum. However, a significant amount of the released miRNA is bound to argonaute proteins like Ago2 or other RNA-binding proteins and is protected from degradation. Within exosomes and other extracellular vesicles (EVs), both miRNAs and mRNAs are well protected by a lipid bilayer and are thus relatively stable (see Figure 7).

These cell-free circulating RNAs, detectable in all body fluids, can function as biomarkers. However, RNA purification from serum/plasma and other biofluids is challenged by low abundances of RNA and high amounts of RNases and inhibitors. Thus, efficient and dedicated purification methods addressing these challenges are needed to accurately assess cell-free RNA molecules.

Learn more at **qiagen.com/LiquidBiopsy**

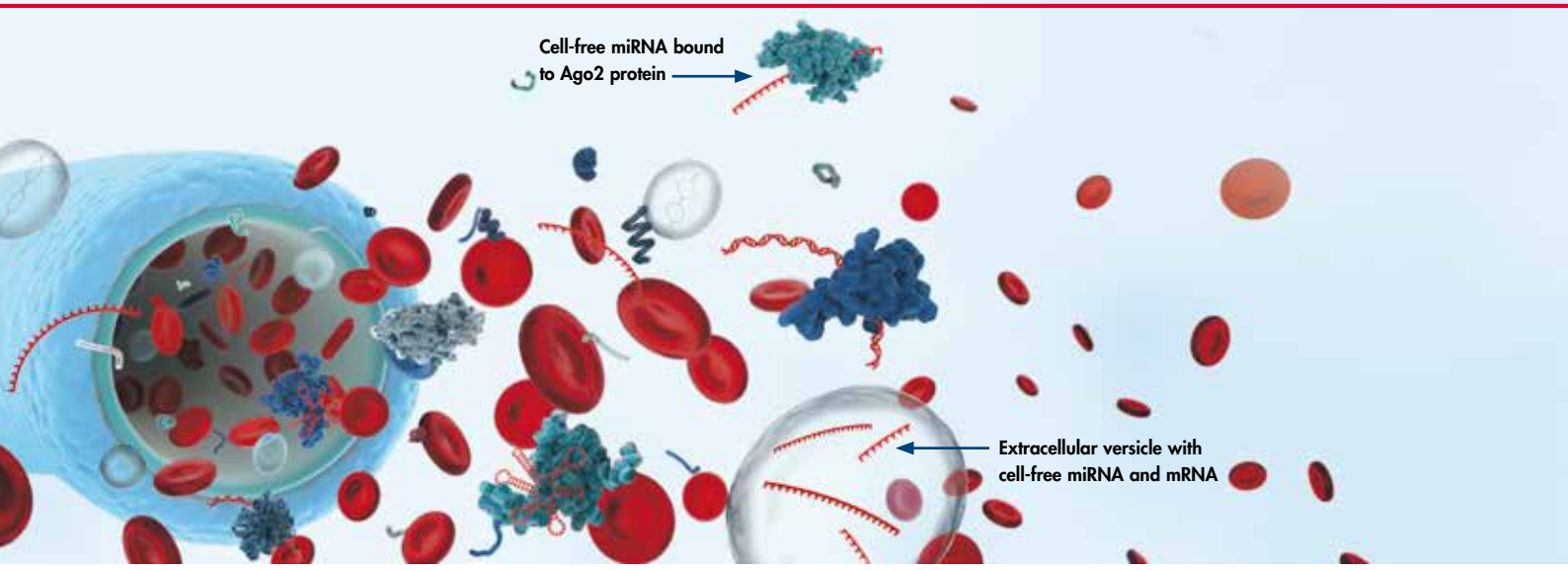

Figure 7. Illustration of circulating cell-free RNA in serum or plasma.

The miRNeasy Serum/Plasma Advanced Kit efficiently purifies total cell-free RNA including miRNA from serum and plasma from only 200 µl of starting sample. The phenol-free protocol uses UCP (ultra-clean production), and MinElute® spin columns allow for small volumes of ultra-clean eluates. Lysis of extracellular vesicles allows analysis of RNA both inside and outside of vesicles. The exoRNeasy Serum/Plasma Kits specifically isolate vesicular RNA using spin-column-based technology.

Removal of genomic DNA contamination

Generally, additional removal of genomic DNA (gDNA) is not required with RNeasy and miRNeasy kits, because a significant portion of gDNA is already removed during RNA purification due to the RNeasy buffer composition or QIAzol® sample pretreatment to lyse and remove gDNA. However, trace amounts of gDNA in an RNA sample can compromise the accuracy of sensitive applications such as real-time RT-PCR. Several technologies can be applied to remove remaining traces of gDNA in your RNA sample (see Figure 8).

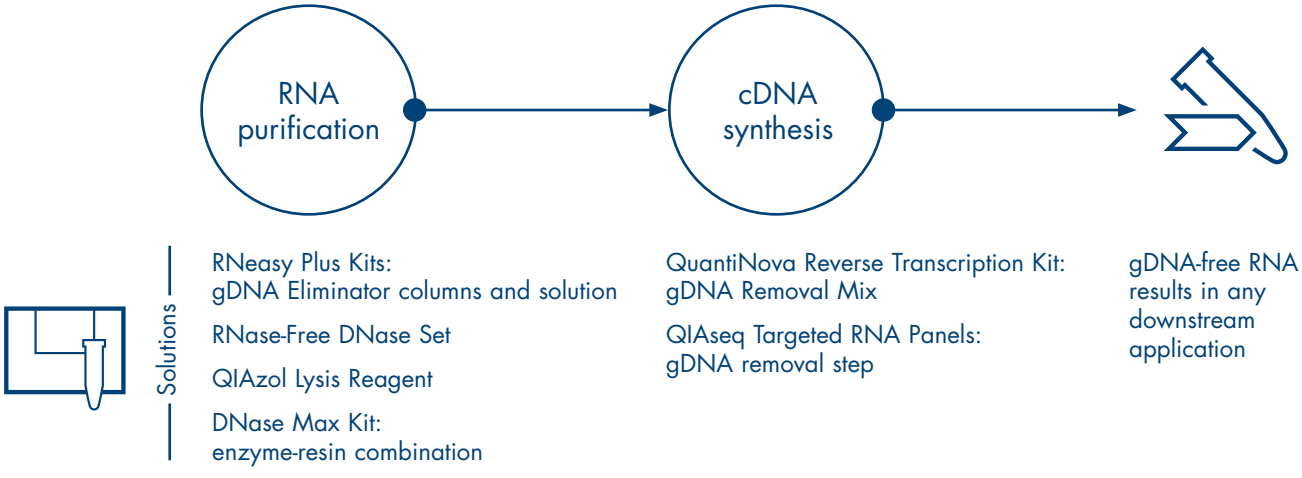

Figure 8. Overview of QIAGEN gDNA removal technologies.

Co-purification of RNA and other analytes

In research areas such as systems biology that involve parallel analysis of different analytes, reliable correlation of data is only possible if the analytes are all purified from the same sample and even the same section from the sample. AllPrep® Kits provide a single, streamlined protocol to purify RNA and other analytes like DNA or proteins from a single sample, with each biomolecule type eluted in a separate fraction or tube. Refer to Table 10 to determine which AllPrep kit is the best solution for your particular application.

With AllPrep kits, there is no need to divide the sample for separate purification procedures, so maximal yields of each biomolecule are obtained (Figure 9). The kits can also be combined with Allprotect® Tissue Reagent, which stabilizes DNA, RNA and proteins to ensure reliable genomic, transcriptomic and proteomic analyses.

Co-purification of DNA and RNA from serum or plasma samples

The QIAamp® ccfDNA/RNA Kit allows co-purification of cell-free DNA and RNA from the same sample, providing excellent yield and quality of both analytes in the same eluate. This allows you to analyze both DNA and RNA mutations from the same liquid biopsy sample or to boost detection sensitivity to discover low-frequency tumor mutations.

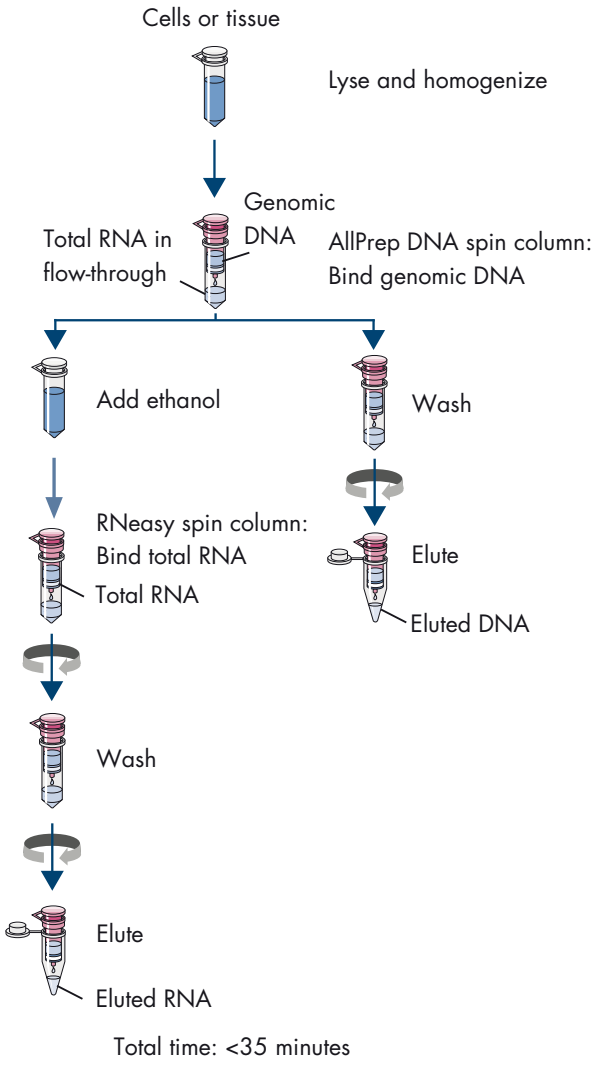

Figure 9. Example of the AllPrep procedure. Different analytes isolated from a sample are eluted in two different eluates.

Purification of viral nucleic acids

A major challenge in purifying viral RNA and DNA from plasma and serum is concentrating the nucleic acids, which may be extremely diluted in a large sample volume. QIAamp virus kits enable purification of viral nucleic acids from starting volumes as low as 140 µl and as high as 10 ml (see Table 11). Viral RNA and DNA are isolated on a QIAamp silica-membrane spin column, efficiently washed and then eluted. QIAamp virus kits use state-of-the art technologies to concentrate viral nucleic acids in plasma and serum samples, followed by nucleic acid purification.

The purified nucleic acids perform well in sensitive downstream applications. The procedure provides increased sensitivity in viral-load monitoring and other applications in which high recovery of viral nucleic acids is essential (Table 4).

		HIV-1 RNA recovered in first elution	
HIV-1 sample (copies/ml)	Theoretical number of HIV-1 copies in sample	% Recovery (mean ±SD)	Number of copies recovered
650	91	96 ± 4.6	87
1300	182	98 ± 2.8	178
13,000	1820	98 ± 1.4	1780
130,000	18,200	95 ± 1.0	17,290
1,000,000	140,000	91 ± 2.8	126,800

Table 4. Example of viral RNA recovery. To determine viral RNA recovery, 140 µl acid citrate dextrose plasma samples with known HIV-1 copy numbers were applied to QIAamp spin columns. A modified protocol involving two elutions was used to determine the efficiency of the spin columns. HIV RNA was detected by an RT-PCR chemiluminescent assay (data excerpted from Lin, H.J., Twandee, T. and Hollinger, F.B. (1997) J. Med. Virol. **51**, 56.

Automate your RNA isolation

Routine RNA purification and handling large numbers of samples is tedious and time-consuming, and human error can introduce variations in RNA yield and quality. QIAGEN's range of automated RNA purification systems supports low to high throughputs — from just a few samples to 96 samples per run. Thorough testing with proven QIAGEN chemistries ensures reproducible purification of high-quality RNA. Dedicated platform-specific kits are available for automated RNA purification (see Table 9 on page 19).

Plus, QIAGEN Instrument Services offers several benefits to help you get the most from your instruments:

- Flexible service support agreements
- Dedicated application support
- Tailor-made training programs

	Low throughput	Medium to high throughput
Spin column-based	**QIAcube®** • Mimics manual spin-column procedure for up to 12 samples per run • Includes a complete workstation with a centrifuge and shaker in minimal space • Automation of almost all manual RNeasy and miRNeasy kits 	**QIAcube HT** • Silica-membrane technology in 96-well format • Processes 24–96 samples per run • Dedicated RNeasy kit for isolation of total RNA, including miRNA or RNA >200 bp to enrich for mRNA 
Magnetic bead-based	**EZ1® Advanced XL instruments** • Magnetic-particle technology to isolate up to 14 samples per run • Full process documentation • RNA isolation from cells and tissues using dedicated EZ1 RNA Kit 	**QIAsymphony® SP and AS** • Magnetic bead-based isolation technology • Assay setup also possible with AS module • Full process documentation • Process up to 96 samples per run, in batches of up to 24 • RNA isolation from cells, tissues and FFPE with one QIAsymphony RNA Kit and predefined protocols • Automated processing directly from primary tubes with PAXgene RNA system 

For more information on QIAGEN automation solutions contact your QIAGEN sales person or visit **www.qiagen.com/automation**.

RNA quality control

Reliable results and accurate quantification in downstream assays depend on the quality of the RNA sample. Thus, RNA quality control (QC) is an integral part of every gene expression experiment and consists of three key parameters:

- **Quantity:** Is there enough RNA to assay?
- **Purity:** Is the RNA free of contaminants?
- **Integrity:** Is the RNA degraded?

The QIAxpert® and QIAxcel® Advanced Systems enable rigorous RNA quality control checks of RNA concentration, purity and integrity with just a few simple steps (Figure 11).

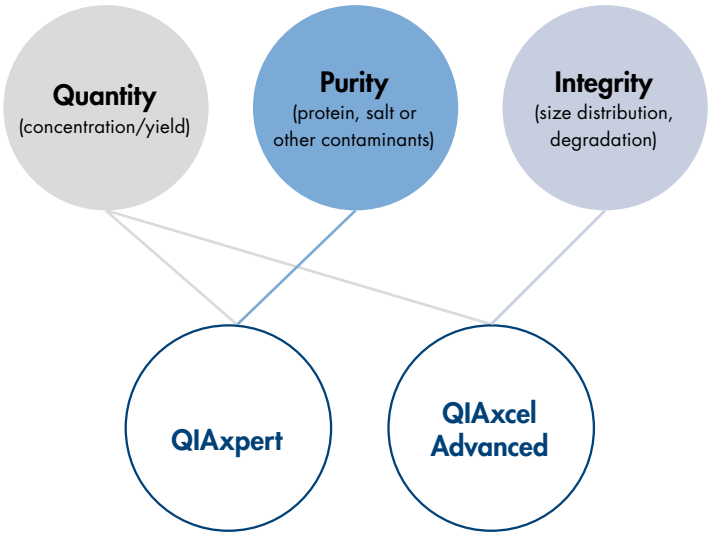

Figure 11. QIAGEN RNA quality control solutions.

Measurement of RNA quantity and purity

RNA concentration is typically determined by measuring its absorbance at 260 nm (A_{260}) in a spectrophotometer. At neutral pH, an absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml. If necessary, dilute samples in a low-salt buffer with neutral pH (e.g., 10 mM Tris·Cl, pH 7.0). To ensure accurate measurements, remove RNases from the cuvettes by washing with a solution of 0.1 M NaOH and 1 mM EDTA and rinsing with RNase-free water. Use the buffer in which the RNA is diluted to zero the spectrophotometer.

The ratio of the absorbance at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the RNA purity with respect to contaminants such as protein. However, absorbance is influenced considerably by pH, and since water is not buffered, the pH and 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 ratios, we recommend measuring absorbance in a low-salt buffer with slightly alkaline pH (e.g., 10 mM Tris·Cl, pH 7.5). An A_{260}/A_{280} ratio of 1.8–2.1 at pH 7.5 is widely accepted as indicative of highly pure RNA. Pure RNA should also yield an A_{260}/A_{230} ratio of around 2; however, there is no consensus on the acceptable lower limit of this ratio. Also, it has not been fully established which contaminants contribute to a low A_{260}/A_{230} ratio. In our experience, increased absorbance at 230 nm in RNA samples is almost always due to contamination by guanidine thiocyanate, a salt that absorbs very strongly at 220–230 nm and can be present at very high concentrations in the lysis buffer or extraction reagent (e.g., TRIzol®) used in most RNA purification procedures. However, concentrations of guanidine thiocyanate of up to 100 mM in an RNA sample do not compromise the reliability of real-time RT-PCR, even when using PCR chemistries that are sensitive to inhibitors.

The QIAxpert is an innovative, microfluidic UV/Vis spectrophotometer that uses reference spectra of known contaminants and state-of-the-art software algorithms to perform a deconvolution of measured UV/Vis spectra according to the Beer–Lambert law for mixtures. This feature, known as Spectral Content Profiling (SCP), allows dye-free and easy differentiation between DNA, RNA and other UV/Vis-absorbing contaminants in complex biological samples.

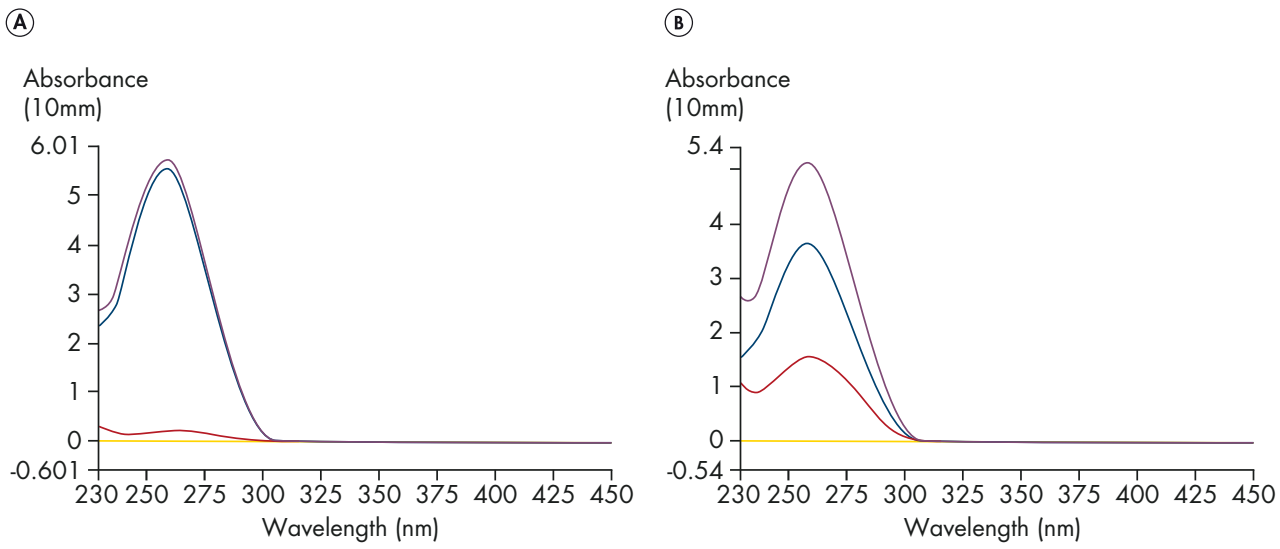

Figure 11. Spectral content profiling discriminates components of complex samples. A A pure sample of RNA shows no significant contamination, evidenced by the simple spectrum. **B** A sample of RNA spiked with gDNA is accurately resolved into the contributing blue absorbance line for RNA and red line for the spiked DNA.

Measuring RNA integrity

The electrophoretic signatures of RNA samples directly correlate with their integrity and quality, which can be compromised through sample processing and nuclease contamination. Thus, integrity and size distribution of total RNA can be checked by denaturing agarose gel electrophoresis, ethidium bromide staining or capillary electrophoresis systems like the QIAxcel Advanced. Capillary electrophoresis enables rapid size-based separation of nucleic acids and ensures good resolution, sensitivity and cost per sample.

RNA samples have unique electrophoretic signatures, which encompass the abundant RNA subpopulations corresponding to the 5S, 18S and 28S rRNA subunits as well as the areas between these major peaks corresponding to the distribution of all other RNA types like mRNAs or regulatory RNAs. The respective expected ribosomal bands of the sample (see Table 5) should appear as sharp bands on the stained gel or the analysis software (Figure 12). If the ribosomal bands in a given lane are not sharp, but appear as a smear of smaller sized RNAs, it is likely that the RNA sample suffered major degradation.

Table 5. rRNA sizes from various sources

Source	rRNA	Size (kb)
E. coli (bacteria)	16S	1.5
	23S	2.9
S. cerevisiae (yeast)	18S	2.0
	26S	3.8
Mouse	18S	1.9
	28S	4.7
Human	18S	1.9
	28S	5.0

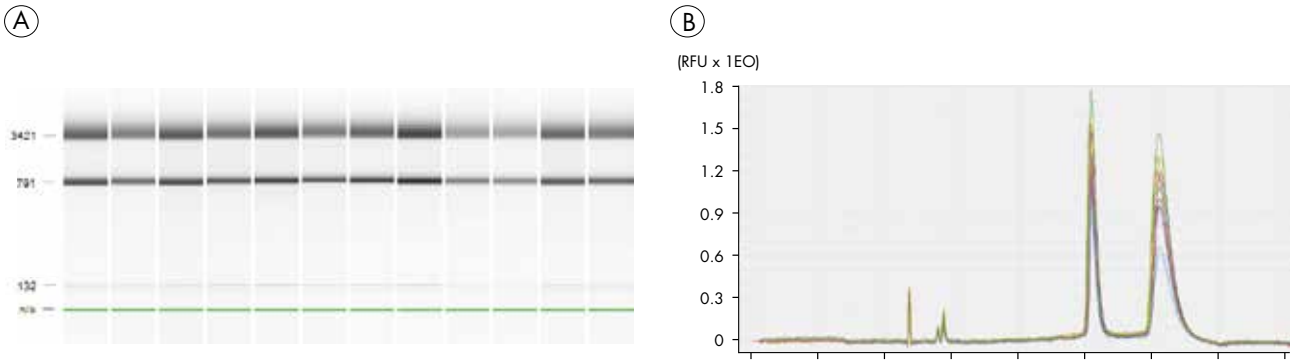

Figure 12. Analysis of total RNA purified from S. pombe. Results presented as **A** a gel image and **B** a superimposed electropherogram view.

Quality indicators such as the RIS (RNA Integrity Score, QIAGEN) or the RIN (RNA Integrity Number, Agilent Technologies) allow objective assessment of RNA sample integrity and standardization of sample quality. RIS and RIN algorithms analyze different electropherogram features, such as peak intensities, signal areas and ratios, including 28S/18S ratio, and compute a value ranging from 1–10. Values >8 correspond to high-quality RNA samples. In many cases, particularly with tissue samples, RNA integrity is mainly influenced by how well the original sample was preserved. Thus, RIS/RIN values close to 10 often cannot be obtained, but sample quality might still be sufficient for downstream analysis.

Product selection guide

Sample collection and stabilization kits

Table 6. Products for sample collection and stabilization.

Sample type	RNA stabilization/collection	RNA stabilization and purification	Stability
Tissues	RNA*later* RNA Stabilization Reagent (for RNA stabilization) cat. nos. 76104, 76106 RNA*later* TissueProtect Tubes (for tissue collection and RNA stabilization) cat. nos. 76163, 76154 Allprotect Tissue Reagent (for DNA/RNA/protein stabilization) cat. no. 76405	RNeasy Protect Mini Kit cat. nos. 74124, 74126	**Allprotect-stabilized samples:** 7 days at 15–25°C 12 months at 2–8°C Longer periods at –20 to –80°C **RNA*later*-stabilized samples:** 7 days at 15–25°C 4 weeks at 2–8°C Longer periods at –20 to –80°C
Mammalian cells	RNAprotect® Cell Reagent (for RNA/DNA stabilization) cat. no. 76526	RNeasy Protect Cell Mini Kit (for RNA/DNA stabilization and RNA purification) cat. no. 74624	7 days at 15–25°C 4 weeks at 2–8°C Longer periods at –20 to –80°C
Bacteria	RNAprotect Bacteria Reagent (for RNA stabilization) cat. no. 76506	RNeasy Protect Bacteria Mini Kit (for RNA stabilization and purification) cat. no. 74524	3 hours at 15–25°C 2 weeks at –20°C 4 weeks at –70°C
Saliva	RNAprotect Cell Reagent (for RNA/DNA stabilization) cat. no. 76526	RNeasy Protect Saliva Mini Kit (for RNA/DNA stabilization and RNA purification) cat. no. 74324	14 days at 15–25°C 4 weeks at 2–8°C Longer periods at –20 to –80°C
Animal whole blood	RNAprotect Animal Blood Tubes (for blood collection and RNA stabilization) cat. no. 76554	RNeasy Protect Animal Blood Kit (for RNA purification) cat. no. 73224	48 hours at 15–25°C 2 weeks at 2–8°C At least 3 months at –20 to –80°C
Human bone marrow	PAXgene Bone Marrow RNA Tubes (for stabilization, transport, and storage of intracellular RNA in bone marrow aspirate samples) cat. no. 764114	PAXgene Bone Marrow RNA Kit (for purification of intracellular RNA from bone marrow samples in PAXgene Bone Marrow RNA Tubes) cat. no. 764133	Up to 3 days at room temperature (15–25°C) Up to 5 days at 2–8°C Long-term at –20 to –80°C
Human tissue / PAXgene Tissue fixed (PF) or PAXgene Tissue fixed, paraffin-embedded (PFPE) tissues	PAXgene Tissue FIX Containers and PAXgene Tissue STABILIZER Concentrate (for collection, fixation and stabilization of tissues and preservation of histomorphology and nucleic acids) cat. nos. 765312, 765512	PAXgene Tissue RNA/miRNA Kit (for isolation and purification of total RNA from tissues fixed and stabilized using the PAXgene Tissue Containers) cat. no. 766134	Up to 7 days at room temperature (15–25°C) Up to 4 weeks at 2–8°C Long-term storage at –20 to –80°C*

* Long-term storage studies are ongoing.

† Purifies total RNA that includes miRNA and other small RNAs.

RNA isolation kits for research use only

Table 7. RNA isolation selection guide

Sample type / application	RNA > 200 nucleotides, enriched for mRNA, lncRNA and other long RNAs	Total RNA including miRNA and other small RNAs
Animal/human cells or easy-to-lyse tissues (e.g., kidney, liver, spleen) and yeast	RNeasy Mini, Midi and Maxi Kits cat. nos. 74104, 74106, 75142, 75162 RNeasy Plus Mini Kits (incl. gDNA eliminator columns) cat. nos. 74134, 74136	miRNeasy Mini Kit cat. no. 217004 RNeasy Plus Mini Kits (supplementary protocol only available for animal/human cells) cat. nos. 74134, 74136
Low biomass/Microsamples samples of animal/human cells and tissues (e.g. fine-needle aspirates, laser-microdissected samples)	RNeasy Micro Kit cat. no. 74004 RNeasy Plus Micro Kit (incl. gDNA eliminator columns) cat. no. 74034 RNeasy UCP Micro (incl. ultra-clean columns and buffers) cat. no. 73934	miRNeasy Micro Kit cat. no. 217084
Fiber- or lipid-rich tissues (e.g., muscle, brain, skin)	RNeasy Plus Universal Kits cat. nos. 73404, 73442 RNeasy Fibrous Tissue Kits cat. no. 74704 RNeasy Lipid Tissue Kits cat. no. 74804	miRNeasy Mini Kit cat. no. 217004 RNeasy Plus Universal Kits cat. nos. 73404, 73442
Plants and fungi	RNeasy Plant Mini Kit (incl. QIAshredder for homogenization) cat. no. 74904	
FFPE tissue slices or PAXgene fixed tissues	RNeasy FFPE* Kit cat. no. 73504 PAXgene Tissue RNA/miRNA Kit (for tissues fixed and stabilized in PAXgene Tissue Containers) cat. no. 766134	miRNeasy FFPE Kit cat. no. 217504 PAXgene Tissue RNA/miRNA Kit (for tissues fixed and stabilized in PAXgene Tissue Containers) cat. no. 766134
Serum/Plasma		miRNeasy Serum/Plasma Advanced Kit cat. no. 217204 exoRNeasy Serum/Plasma Kits (for exosomal RNA isolation) cat. nos. 77023, 77044, 77064
Animal blood (stabilized)	RNeasy Protect Animal Blood Kit cat. no. 73224	RNeasy Protect Animal Blood Kit + Buffer RWT cat. nos. 73224, 1067933
Human blood (stabilized in PAXgene Blood RNA tubes) for research applications	See Table 12	PAXgene Blood miRNA Kit cat. no. 763134
Human blood (fresh, anticoagulant-stabilized)	QIAamp RNA Blood Mini Kit cat. no. 52304	miRNeasy Mini Kit (requires Buffer EL in addition) cat. no. 217004, 79217
RNA cleanup and concentration from enzymatic reactions or crude RNA preparation	RNeasy MinElute Cleanup Kit cat. no. 74204	
RNA cleanup from enzymatic reactions or crude RNA preparation	RNeasy Mini, Midi and Maxi Kits cat. nos. 74104, 74106, 75142, 75162	

* Since RNA in FFPE material can be heavily degraded, the RNeasy FFPE Kit isolates total RNA >70 nucleotides to also capture fragmented RNA.

Table 8. Kits for manual, high-throughput RNA purification

Sample type	RNA >200 bp, enriched for mRNA and lncRNA	Total RNA incl, miRNA and other small RNAs
Cells and easy-to-lyse tissues	RNeasy 96 and RNeasy 96 Plus Kits cat. nos. 74181, 74182, 74192	miRNeasy 96 Kit cat. no. 217061
Difficult-to-lyse tissues (e.g., muscle, brain)	RNeasy 96 Universal Tissue Kit cat. no. 74881	
Human Blood (stabilized)	PAXgene 96 Blood RNA Kit cat. no. 762331	
RNA cleanup	RNeasy 96 Kit cat. no. 74181	

Table 9. Automated RNA purification kits

Automation platform	Sample type	RNA > 200 bp, enriched for mRNA and lncRNA	Total RNA including miRNA and other small RNAs
QIAcube	All sample types	Most of our RNA purification kits can be automated on the QIAcube. For more information, please browse the QIAcube Standard Protocols	
QIAcube HT	Animal/ human tissue Animal/ human cells	RNeasy 96 QIAcube HT Kit cat. no. 74171	RNeasy 96 QIAcube HT Kit cat. no. 74171
EZ1 Advanced	Animal/ human tissue	EZ1 RNA Tissue Mini Kit cat. no. 959034	
QIAsymphony SP	PAXgene stabilized human blood Animal cells and tissues incl. FFPE	QIAsymphony PAXgene Blood RNA Kit cat. no. 762635 QIAsymphony RNA Kit cat. no. 931636	QIAsymphony PAXgene Blood RNA Kit cat. no. 762635 QIAsymphony RNA Kit cat. no. 931636

Co-purification of RNA and other analytes

Table 10. Kits for co-purification of RNA and other analytes

Application	Kit name	Max. sample amount
DNA and RNA from limited amounts of cells and tissue	AllPrep DNA/RNA Micro Kit* cat. no. 80284	Up to $5x10^5$ cells or 5 mg tissue
DNA and RNA from the same sample of cells and tissue	AllPrep DNA/RNA Mini Kit* cat. no. 80204	Up to $1x10^7$ cells or 30 mg tissue
DNA and RNA, incl. miRNAs, from FFPE material	AllPrep DNA/RNA FFPE Kit cat. no. 80234	Up to 4x10 µm sections or 2x20 µm sections
RNA and Proteins from cells and tissue	AllPrep RNA/Protein Kit cat. no. 80404	N/A
DNA, RNA and Proteins from cells and tissue	AllPrep DNA/RNA/Protein Mini Kit* cat. no. 80004	Up to $1x10^7$ cells or 30 mg tissue
High-throughput DNA and RNA isolation	AllPrep DNA/RNA 96 Kit* cat. no. 80311	Up to $1x10^7$ cells or 30 mg tissue
DNA and RNA, incl. miRNA from cells and difficult-to-lyse tissue (e.g., fiber- or lipid-rich tissues)	AllPrep DNA/RNA/miRNA Universal Kit* cat. no. 80224	Up to $1x10^7$ cells or 30 mg tissue
DNA and RNA incl. miRNA from serum or plasma in the same eluate	QIAamp ccfDNA/RNA Kit cat. no. 55184	Up to 4 ml of plasma or serum

* Kit can be used in combination with Allprotect Tissue Reagent, which stabilizes DNA, RNA and protein in tissues.

Viral nucleic acid isolation

Table 11. QIAamp solutions for isolation of viral nucleic acids.

Starting material	Nucleic acids purified	Kit	Max. sample amount	Elution volume	Concentration factor
Human plasma, serum and CSF	Viral RNA	QIAamp Viral RNA Mini Kit cat. nos. 52904, 52906	140 µl	60 µl	2
Human plasma, serum, CSF and other body fluids	Viral RNA and DNA	QIAamp MinElute Virus Spin Kit cat. no. 57704	200 µl	20–150 µl	10
		QIAamp MinElute Virus Vacuum Kit cat. no. 57714	500 µl		25
Human plasma, serum, CSF, urine and other body fluids	Viral RNA and DNA	QIAamp Circulating Nucleic Acid Kit cat. no. 55114	1–5 ml	20 µl	200–500

Kits for in vitro diagnostic applications

Table 12. Solutions for in vitro diagnostic applications.

Starting sample material	Description	Kit	Details
Human whole blood	Collection, transport and storage of blood and stabilization of intracellular RNA for molecular testing	PAXgene Blood RNA Tubes cat. no. 762165	Stability: Up to 3 days at room temperature (15–25°C) Up to 5 days at 2-8°C 11 years* at –20°C or –70°C
Human blood (stabilized in PAXgene Blood RNA tubes)	Purification of intracellular RNA from whole blood to be used in in vitro diagnostic (IVD) tests	PAXgene Blood RNA Kit‡ cat. no. 762164 (US and Canada), 762174 (other countries)	Isolates RNA >200 nucleotides, enriched for mRNA, lncRNA and other long RNAs
FFPE tissue slices or PAXgene fixed tissues	Purification of total RNA from formalin-fixed paraffin embedded (FFPE) tissue sections for in vitro diagnostic use	RNeasy DSP FFPE† cat. no. 73604	Isolates RNA, enriched for mRNA, lncRNA and other long RNAs

* Long-term study of blood storage in PAXgene Blood RNA Tubes is ongoing.

† Since RNA in FFPE material can be heavily degraded, the RNeasy DSP FFPE Kit isolates total RNA >70 nucleotides to also capture fragmented RNA.

‡ Kit is automatable on QIAcube; not available in all countries; please inquire.

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