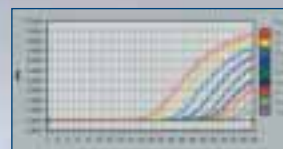
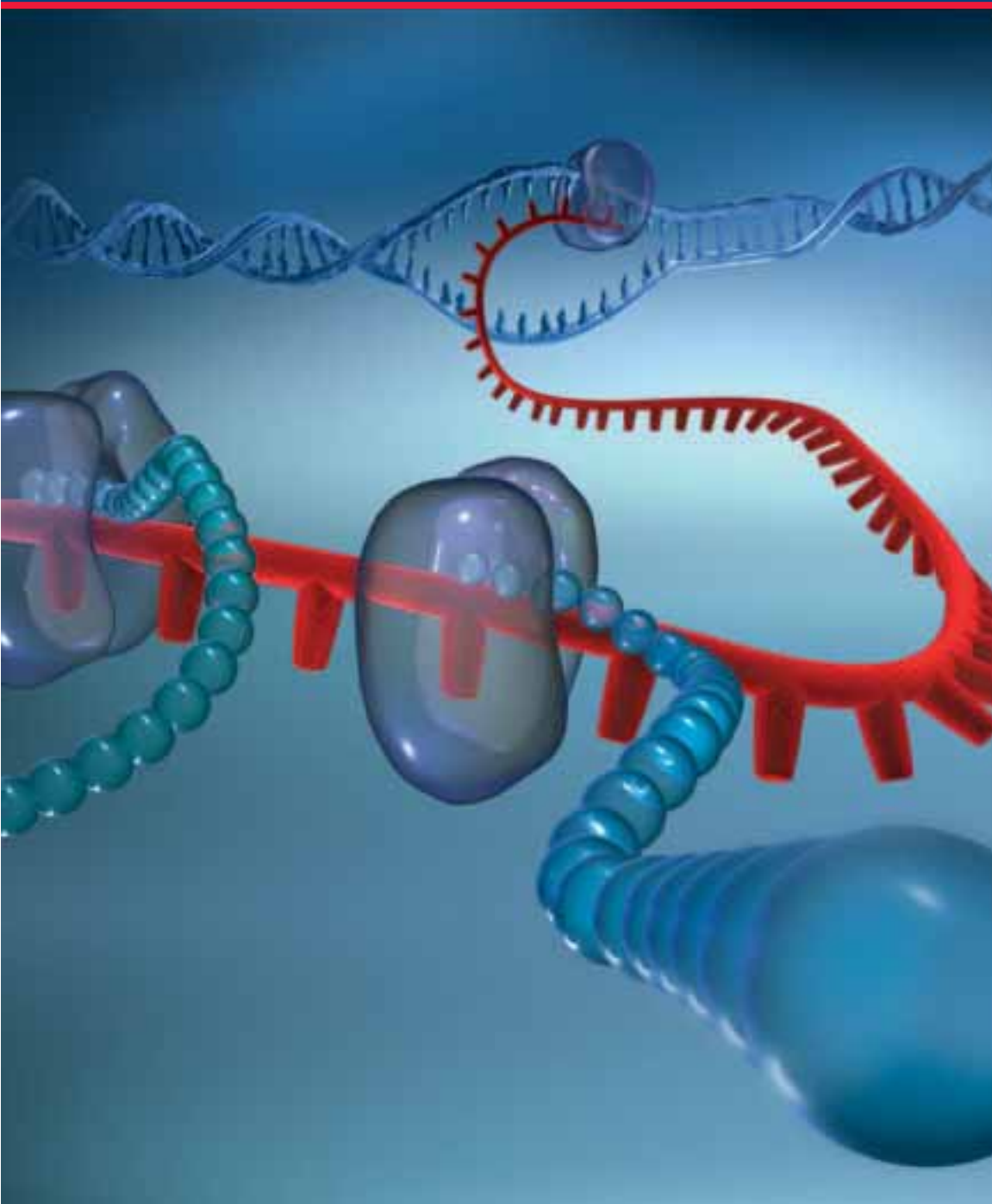


Integrated Solutions — Gene Expression Analysis

High-Performance RNA for Gene Expression Analysis



1. Introduction	4
2. Important considerations before starting	5
2.1 Size is important — is the RNA purification procedure appropriate for the amount of starting material?	5
2.2 Not all samples are alike — don't they deserve a suitable method?	5
2.3 More samples faster — is it time for high-throughput RNA purification?	6
2.4 Tissue management — how can processing of tissue samples be made easier?	7
2.5 Getting optimal results in gene expression analysis — are the upstream procedures appropriately optimized?	7
2.6 mRNA or total RNA — when is the part better than the whole?	8
2.7 Integrated solutions for gene expression analysis — QIAGEN products for preparing RNA	8
3. Sample collection and stabilization	9
3.1 Stabilization of animal tissues	10
3.2 Stabilization of whole blood	11
3.3 Stabilization of bacteria	11
4. Sample disruption and homogenization	12
4.1 Low-throughput homogenization	13
4.2 High-throughput disruption and homogenization	14
5. Purification of RNA	15
5.1 QIAGEN systems for RNA purification	17
5.2 Purification of total RNA from small samples	18
5.3 Purification of total RNA from cells	19
5.4 Purification of total RNA from tissues	20
5.5 Purification of total RNA from whole blood	22
5.6 Purification of total RNA from plants and fungi	24

5.7	Purification of total RNA from bacteria	25
5.8	Purification of small RNA or both total RNA and genomic DNA	26
5.9	Purification of poly A ⁺ mRNA	27
5.10	Cleanup and concentration of RNA	27
5.11	High-throughput and/or automated purification of RNA	28
5.12	DNase digestion during RNA purification	34
6.	Ordering Information	35

To get accurate results in gene expression analysis, it is important to have effective methods for obtaining RNA from biological samples. Getting high-performance RNA requires care in a process that starts with sample collection and stabilization and continues with sample disruption and homogenization and RNA purification. Each step of this process is important:

- **Sample collection and stabilization** — it is essential that the in vivo gene expression profile is preserved, otherwise gene expression analysis results will be inaccurate
- **Sample disruption and homogenization** — to ensure efficient isolation of RNA during the purification procedure, RNA must be completely released from the sample and the viscosity of the lysed sample must be reduced
- **RNA purification** — since each downstream assay has its own requirements with regard to quality and quantity of RNA, it is important that the RNA purification procedure can purify RNA at the amount and concentration required and without chemical contaminants that can affect the assay

This brochure discusses important issues to be considered for each of these three steps (pages 5–8). It then goes on to describe optimized methods from QIAGEN for each step (pages 9–34). These optimized methods are fully integrated with each other, ensuring streamlined workflows and high-performance RNA for gene expression analysis.

Workflow for Gene Expression Analysis



In addition to products for preparing RNA, QIAGEN offers a range of other products for gene expression analysis. These include products for:

- **Reverse transcription** — Omniscript® and Sensiscript® RT Kits
- **One-step RT-PCR** — QIAGEN® OneStep RT-PCR Kit
- **Quantitative, real-time RT-PCR** — QuantiTect® Kits and Assays
- **RNA interference** — siRNAs and RNAiFect™ Transfection Reagent

To find out more, visit www.qiagen.com or contact your local QIAGEN office or distributor.

2.1 Size is important — is the RNA purification procedure appropriate for the amount of starting material?

Modern sample-collection methods, such as cell sorting by FACS®, laser microdissection (LMD), and fine-needle aspiration (FNA), allow isolation of single cells or very small amounts of tissue. With these small samples, it is important to use an efficient RNA purification method, so that sufficient RNA can be obtained for gene expression analysis. Highly sensitive analysis techniques, such as real-time RT-PCR, are capable of detecting the small amounts of target RNA in these samples.

For other applications, it is often necessary to use relatively large amounts of starting material or highly concentrated RNA. RNA purification from large samples provides a pool of RNA that can be used for multiple experiments. Large amounts of starting material are also required in order to get significant yields of RNA from samples that have low RNA content or are difficult to process, such as adipose and skin tissues and trace forensic samples.

Before starting RNA purification, it is therefore important to determine the amount of starting material that will be available and/or to decide the amount and concentration of purified RNA that is required. A method that enables RNA purification within these specifications should then be selected.

2.2 Not all samples are alike — don't they deserve a suitable method?

Biological materials vary in their properties. Some sample sources can cause problems in RNA purification and analysis due to their properties (e.g., RNA amount, cell structure, or presence of certain substances). These sample sources require special considerations, which are generally not necessary when working with “standard” sample sources (e.g., cell cultures, liver, and kidney).

Cultured animal cells are perhaps the least diverse and most “standard” sample sources. Cells can vary in the amount of RNA or RNases per cell, but are otherwise generally handled similarly in RNA purification.

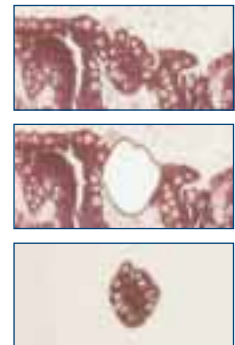
Animal tissues, however, show more variability. “Standard” soft tissues, such as kidney or liver, generally require harsher treatment than animal cells during disruption and homogenization.

More difficult tissues may require specialized methods for RNA purification. Fatty tissues, for example, require stringent lysis to remove fats that can interfere with purification. Also, contractile proteins, connective tissue, and collagen can interfere with purification of RNA from fibrous tissues. Proteinase digestion or strong organic solvents are often used with these sample types to help release RNA.

Blood contains a number of enzyme inhibitors that can interfere with downstream RNA analysis. In addition, common anticoagulants, such as heparin and EDTA, can interfere with downstream assays. RNA purification from blood therefore requires a method that provides high-quality RNA without contaminants or enzyme inhibitors.

Plant material presents special challenges for RNA purification, since several plant metabolites have chemical properties similar to those of nucleic acids. These metabolites are difficult to remove during the RNA purification procedure and can affect downstream assays. In addition, plant lysates are usually viscous, making them difficult to pipet. Commonly used techniques often require

Laser Microdissection



Cultured Cells



Animal Tissues



adaptation before they can be used with plant samples. RNA purification is often improved by adjusting growth conditions, but this can also be counter-productive, since gene expression analysis is often concerned with the effects of growth conditions.

Bacteria have mRNAs that differ from eukaryotic mRNAs in a number of essential features. Prokaryotic mRNAs have no 5' cap and only rarely have poly-A tails. The absence of a poly-A tail means that mRNA isolation by hybrid selection is not possible. Also, some bacterial mRNAs are highly unstable. Since mRNAs are very rapidly turned over in bacteria, gene expression studies are more difficult in prokaryotes than in eukaryotes. This makes stabilization of RNA in bacterial cultures especially critical.

When purifying RNA, it is therefore important to use a method that takes into account the properties of the starting material. The method should enable maximum recovery of high-quality RNA without contaminants that can affect downstream assays.

From Low to
High Throughput



2.3 More samples faster — is it time for high-throughput RNA purification?

Research projects, at some stage, need to be scaled up. As successful research accelerates, the demand to process more samples faster leads to the need for high-throughput RNA purification. Low-throughput methods, which deal with small numbers of samples at a time, are not sufficient for many gene expression analysis projects, which require study of large numbers of genes or large numbers of samples. These types of projects are especially important in fields such as drug discovery, where the effect of many different stimuli on a small number of sample types are studied, or environmental and populational studies, where the effect of a similar set of stimuli on a large number of samples are investigated.

When developing a new high-throughput method, it is necessary to start with a reliable low-throughput method and to scale it up. Scaling up a low-throughput method, however, is not trivial. A reliable high-throughput method must achieve results comparable to the existing method. Critical factors to be considered for the high-throughput method include:

- **Reproducibility** — how much does high throughput decrease individual prep-to-prep variation?
- **Handling** — how much does scaling up reduce the hands-on time per prep?
- **Efficiency** — how much do increases in throughput make the whole process more efficient?
- **Costs** — how much time and money are saved by scaling up to high throughput?
- **Bottlenecks** — what rate-limiting steps need to be addressed to streamline the process?

Both manual and automated high-throughput methods have the potential to improve on all of these factors. With the increase in gene expression analysis in the post-genomic era, more and more labs are switching from manual to automated solutions. Automation has the potential to increase standardization, efficiency, and reproducibility, with lower costs per prep and less hands-on time.

2.4 Tissue management — how can processing of tissue samples be made easier?

In preference to cultured cells, tissues are increasingly becoming a focus for gene expression analysis studies. This is because tissues are more similar to “real life” (i.e., to how genes are expressed in a living organism) than cultured cells.

Immediate stabilization of RNA in tissues is necessary because, directly after sample collection, changes in the gene expression pattern occur due to specific and nonspecific RNA degradation as well as to transcriptional induction. Purification of RNA from tissues can be especially problematic because samples must be thoroughly homogenized before RNA purification, and different types of tissues may be more or less difficult to process. These problems can be overcome by using a tissue-management system.

With a tissue-management system, a range of tissue types can be used as starting material and processed simultaneously according to a single protocol that starts with sample collection and ends with RNA purification. A tissue-management system ensures preparation of high-quality RNA that performs well in downstream applications, and saves time and effort by avoiding the need to follow a different protocol for each tissue type and for each stage of the RNA preparation procedure.

TissueLyser System



2.5 Getting optimal results in gene expression analysis — are the upstream procedures appropriately optimized?

RNA quality can have a tremendous effect on downstream assays such as real-time RT-PCR and microarray analysis. Important factors for downstream applications include not only RNA purity and yield, but also how well the RNA reflects the true gene expression profile of the organism. For accurate gene expression analysis, RNA in the sample must be stabilized at sample collection. In unstabilized samples, gene induction or down-regulation triggered by sample manipulation can lead to inaccurate results in downstream analysis.

Since RT-PCR consists of multiple rounds of enzymatic reactions, it is very sensitive to impurities, such as proteins, phenol/chloroform, salts, ethanol, and EDTA. In addition, contamination of RNA with genomic DNA can bias gene expression results, leading to false positives or inaccurate quantification. Contaminants can interfere at different stages of real-time RT-PCR, such as reverse transcription, amplification, and fluorescence detection. Microarray analysis is also affected by RNA purity. Contaminants in the RNA may lead to reduced yields in enzymatic labeling reactions, reduced lengths of labeled cDNA, and high backgrounds.

Reproducible preparation of RNA is important, especially when comparing gene expression in multiple samples. Comparative gene expression analysis is only valid if RNA preparation is highly reproducible, so that sample comparison is not influenced by variations in RNA preparation.

RNA yield is particularly important when performing multiple assays of the same sample or when working with small, valuable samples or starting materials with low amounts of RNA. From these samples, it is important to get relatively high yields so that there is sufficient RNA for downstream analysis. With low concentrations of RNA, larger volumes of template need to be used in downstream reactions, which can increase the effect of any contaminants in the sample. This makes

it important to obtain high yields and/or to concentrate and clean up the RNA before use in downstream assays.

Successful results in the downstream assay also depend on assay setup. For example, in real-time RT-PCR, optimal primers and probes should be designed and used at the appropriate concentrations, RNA should be efficiently and sensitively reverse transcribed, and target cDNA should be specifically amplified. For more information on this topic, please request a copy of *Critical Factors for Successful Real-Time PCR* from your local QIAGEN office or distributor.

2.6 mRNA or total RNA — when is the part better than the whole?

RNA purification in the last 20 years has moved towards more total RNA purification and less purification of mRNA. This is due in part to more specific detection technologies that make purification of mRNA unnecessary. In addition, methods for purifying total RNA have improved over the years, with more streamlined workflows and less chemical contamination. Today, methods for total RNA purification can be faster than those for mRNA purification.

Using total RNA has a number of advantages over using mRNA. Firstly, since there are larger amounts of total RNA per cell than mRNA alone, it is easier to quantify. The large proportion of rRNA also serves as a target for RNases, slowing down nonspecific digestion of mRNA species. Secondly, purification of mRNA introduces a bias against mRNAs with shorter poly-A tails, which is usually a feature of mRNAs from genes with low expression levels.

However, there is still a limited need for mRNA instead of total RNA. For example, in direct cDNA labeling procedures for microarray analysis, it may be advantageous to use mRNA instead of total RNA. Also, cDNA library construction usually starts with mRNA, although total RNA can also be used if, for example, oligo-dT primers are used. SAGE™ technology for genome-wide analysis of gene expression relies on the use of small oligomer primers and therefore requires mRNA.

2.7 Integrated solutions for gene expression analysis — QIAGEN products for preparing RNA

QIAGEN provides solutions for each of the following steps in preparing RNA and analyzing gene expression: sample collection and stabilization, sample disruption and homogenization, RNA purification, and downstream assays. The material generated in one step (e.g., stabilized tissue sample) can be used directly in the subsequent step (e.g., disruption of the tissue sample). QIAGEN offers complete and integrated solutions for gene expression analysis, which will be described in the following sections of this brochure. For more detailed information on our wide range of products for gene expression analysis, please visit www.qiagen.com, or contact your local QIAGEN office or distributor for further literature, such as *Critical Factors for Successful Real-Time PCR*.

Purification of RNA from clinical samples: www.qiagen.com/goto/bro/RNAclinical

Purification of RNA from other samples: www.qiagen.com/goto/bro/RNAother

Cleanup and concentration of RNA: www.qiagen.com/goto/bro/RNAcleanup

Sample collection can occur under relatively controlled conditions, such as in a laboratory or clinic, or in an area that is remote from the site of sample processing and analysis, such as in the field or in an operating room. Sample stabilization is especially important with remote sample collection, since there is a significant delay between collection and processing. However, even with sample collection in the laboratory, changes in gene expression can occur during harvesting, handling, and RNA purification. When collecting a large number of samples, or if appropriate care is not taken, these changes significantly affect gene expression analysis results.

Once a biological sample is harvested, its RNA becomes extremely unstable. Gene induction or down-regulation triggered by sample manipulation can also occur (Figure 1). Since different transcripts are affected differently, these changes will affect gene expression analysis results. Immediate stabilization of the RNA and preservation of the gene expression pattern is critical for accurate gene expression analysis.

General degradation can be assessed by observing the integrity and size distribution of total RNA using denaturing agarose gel electrophoresis and ethidium bromide staining or using an Agilent 2100 bioanalyzer.

A number of different methods exist for stabilization of RNA. These include the use of strong denaturing reagents, rapid freezing, and patented stabilization reagents.

Many RNA purification methods rely on highly denaturing chaotropic agents, such as guanidine isothiocyanate and guanidine hydrochloride, to immediately inactivate RNases and enable purification of intact RNA. However, samples must be disrupted and homogenized in these reagents immediately after harvesting to prevent significant changes in gene expression.

Traditional methods for stabilizing nucleic acids rely on rapid freezing of the sample in liquid nitrogen or on dry ice. These methods require special equipment, such as insulated vacuum flasks, for handling cold materials. An important consideration is how rapidly the sample is frozen. The size of the sample is critical, since larger samples will freeze more slowly, and changes in the RNA expression pattern can occur as the sample is frozen. Both liquid nitrogen and dry ice have a relatively low heat capacity, and even with small samples, freezing is not instantaneous throughout the whole sample. As with addition of strong denaturing reagents, rapid freezing can only be carried out after sample harvest and thus cannot prevent changes during, for example, centrifugation to harvest bacterial cells.

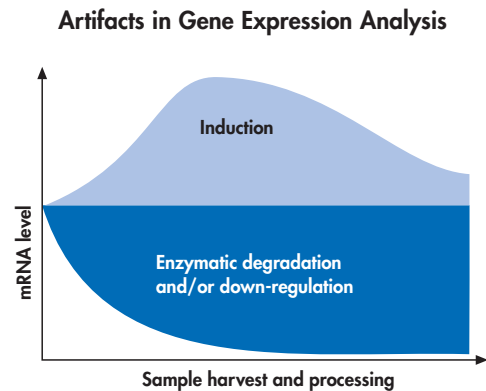


Figure 1 Drastic changes in gene expression profiles can occur when using conventional techniques for RNA purification. These changes are caused by enzymatic degradation of gene transcripts, and gene induction and/or down-regulation.

A major disadvantage of using liquid nitrogen to stabilize nucleic acids is that samples can easily thaw during handling. Therefore, rapid freezing methods are often followed by disruption and homogenization of the sample in strong denaturing reagents, while taking care not to let the sample thaw.

Reagents are available from QIAGEN for stabilizing RNA in tissues, whole blood, and bacteria (see below). These reagents quickly permeate the biological sample to protect the gene expression profile. Samples can be conveniently and safely handled at room temperature without the risk of changes in gene expression.

3.1 Stabilization of animal tissues

RNA^{later}™ RNA Stabilization Reagent — for immediate stabilization of the gene expression profile in harvested tissue

- Immediate RNA stabilization and protection ensures reliable gene expression and gene-profiling data
- Convenient and safe handling at room temperature; no need for liquid nitrogen or dry ice
- Archiving of samples without risk of RNA degradation, even after multiple freeze–thaw cycles

Tissue samples are submerged in an appropriate volume of RNA^{later} RNA Stabilization Reagent immediately after harvesting. The reagent preserves RNA for up to 1 day at 37°C, 7 days at 18–25°C, or 4 weeks at 2–8°C, allowing processing, storage, and shipping of samples without the need for liquid nitrogen or dry ice (Figure 2). Alternatively, the samples can be placed at –20°C or –80°C for archival storage. RNA^{later} TissueProtect Tubes provide premeasured volumes of RNA^{later} RNA Stabilization Reagent in reclosable tubes for convenient handling and sample storage. After stabilization, RNA can be purified using one of QIAGEN's RNeasy® Kits. RNeasy Protect Kits, which combine RNA^{later} and RNeasy technologies, provide stabilization and purification of RNA in the same kit. For details on RNeasy Kits for use with tissue samples, see page 20.

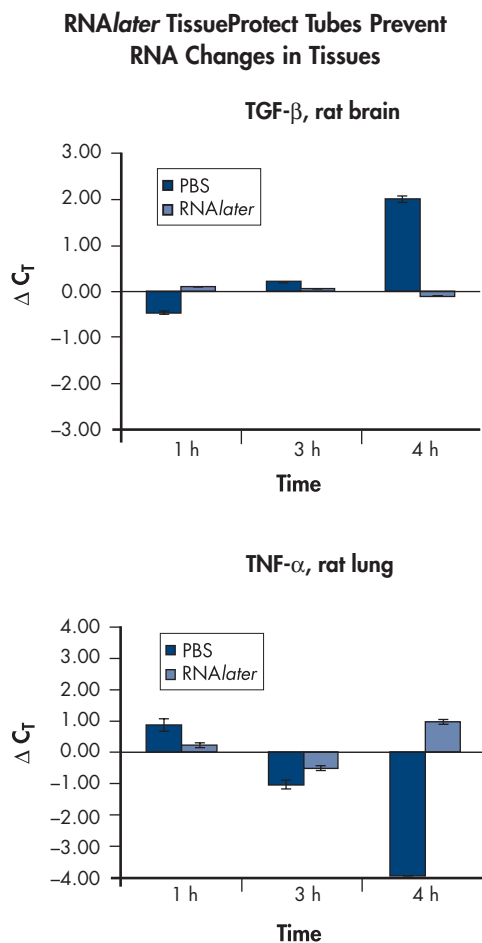


Figure 2 Rat tissues (10 mg each) were excised and stored at room temperature for up to 4 hours in phosphate-buffered saline (PBS) or RNA^{later} RNA Stabilization Reagent (RNA^{later}). At the indicated times, RNA was purified using the RNeasy Protect Mini Kit. Total RNA was used for quantitative RT-PCR using the QuantiTect Probe RT-PCR Kit and primers and probe specific for the transforming growth factor β (TGF-β) gene or the tumor necrosis factor α (TNF-α) gene. Analyses were carried out in triplicate on the ABI PRISM® 7700 Sequence Detection System. The change in threshold cycle (C_t) is shown, relative to the C_t at time zero.

3.2 Stabilization of whole blood

PAXgene™ Blood RNA Tubes enable collection of whole blood samples and immediate stabilization of intracellular RNA. These tubes form part of the PAXgene Blood RNA system, which is an integrated system for collection, stabilization, and purification of RNA from whole blood. For details, see page 22.

3.3 Stabilization of bacteria

RNAprotect™ Bacteria Reagent — for *in vivo* stabilization of the gene expression profile in bacteria

- Immediate RNA stabilization and protection ensures reliable gene expression and gene-profiling data
- Convenient and safe handling at room temperature; no need for liquid nitrogen or dry ice

Two volumes of RNAprotect Bacteria Reagent are added directly to one volume of bacterial culture prior to RNA purification, providing immediate stabilization of RNA (Figure 3). The stabilization allows time for efficient bacterial lysis using a choice of protocols: enzymatic lysis, mechanical disruption, or a combination of both methods. Following stabilization and bacterial lysis, RNA can be purified using an RNeasy Mini, Midi, or Maxi Kit (page 19). RNeasy Protect Bacteria Kits, which integrate RNAprotect stabilization with RNeasy purification, are also available (page 25).

RNAprotect Bacteria Reagent Prevents mRNA Degradation

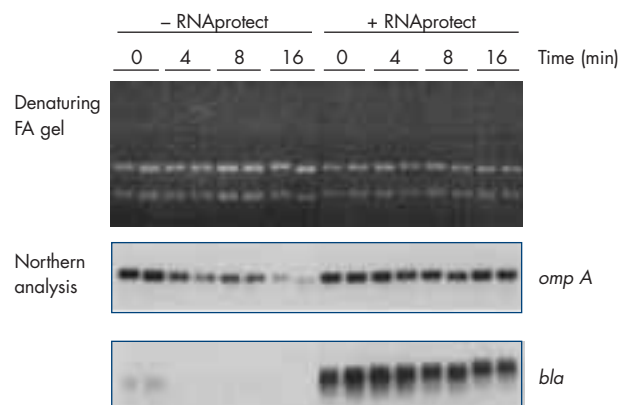


Figure 3 In order to monitor mRNA degradation only, transcription was stopped by adding the RNA polymerase inhibitor rifampicin to a growing culture of *E. coli*. The culture was split into two halves, and RNAprotect Bacteria Reagent was added to one half. Samples were left at room temperature for 0, 4, 8, and 16 minutes before centrifugation and RNA purification. The resulting RNA was analyzed by agarose gel electrophoresis (top panel). Expression of two marker genes with different half lives was examined by northern blot analysis. Middle panel: *ompA* (half life of 15 minutes); bottom panel: β -lactamase (half life of 2–5 minutes).

Efficient disruption and homogenization of the starting material is an absolute requirement for all total RNA purification procedures. Disruption and homogenization are generally carried out in the presence of an organic solvent or a strong chaotropic agent in order to inhibit endogenous RNases that are released during the process. However, changes in the RNA expression pattern can occur prior to or during disruption and homogenization. For accurate gene expression analysis, the sample should be stabilized first (see page 9). Disruption and homogenization are two distinct steps.

Disruption: Complete disruption of plasma membranes of cells and organelles releases all the RNA contained in the sample. Incomplete disruption results in significantly reduced RNA yields.

Homogenization: Homogenization reduces the viscosity of the cell 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 in purification procedures and therefore significantly reduced yields.

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step. Table 1 gives an overview of different disruption and homogenization methods suitable for various starting materials.

Table 1. Disruption and Homogenization Methods for Different Starting Materials

Starting material	Disruption method	Homogenization method
Cultured animal cells	Addition of lysis buffer	Rotor–stator homogenizer or QIAshredder or syringe and needle
Animal tissues	Rotor–stator homogenizer Mortar and pestle Tissuelyser	Rotor–stator homogenizer QIAshredder or syringe and needle Tissuelyser
Bacteria	Enzymatic digestion followed by addition of lysis buffer Tissuelyser with glass beads	Vortexing Tissuelyser with glass beads
Yeast	Enzymatic digestion of cell wall followed by lysis of spheroplasts by addition of lysis buffer Tissuelyser with glass beads	Vortexing Tissuelyser with glass beads
Plants and filamentous fungi	Mortar and pestle Tissuelyser	QIAshredder Tissuelyser

For disruption using a mortar and pestle, the sample is frozen immediately in liquid nitrogen and ground to a fine powder under liquid nitrogen. Lysis buffer is added and then homogenization is carried out using, for example, a syringe and needle. Both cell and tissue lysates can be homogenized using a syringe and needle. As a convenient and safer alternative, QIAGEN offers QIAshredder homogenizers (see page 13).

Rotor–stator homogenizers thoroughly disrupt and simultaneously homogenize animal tissues in the presence of lysis buffer. The time taken is 5–90 seconds, depending on the toughness of the sample. Rotor–stator homogenizers can also be used to homogenize cell lysates. The rotor turns at a very high speed, causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. Rotor–stator homogenization is an effective way of

processing individual samples, but it is not practical for large numbers of samples. The samples must be processed individually, and the rotor–stator homogenizer must be cleaned after each sample to prevent cross-contamination. For simultaneous processing of large numbers of samples, QIAGEN offers the TissueLyser (see page 14).

4.1 Low-throughput homogenization

QIAshredder homogenizer — for simple and rapid homogenization of cell and tissue lysates

- Replaces syringe-and-needle homogenization
- Reduces loss of sample material
- Eliminates cross-contamination between samples
- Filters out insoluble debris and reduces viscosity

Using QIAshredder homogenizers is a fast and efficient way to homogenize cell and tissue lysates without cross-contamination of the samples. The QIAshredder homogenizer consists of a unique biopolymer-shredding system in a microcentrifuge spin-column format. The lysate is loaded onto the QIAshredder homogenizer placed in a collection tube and centrifuged; the homogenized lysate is then collected. In general, similar yields and quality of RNA are obtained as with rotor–stator homogenization (Figure 4).

Comparison of RNA Yields with Different Homogenization Methods

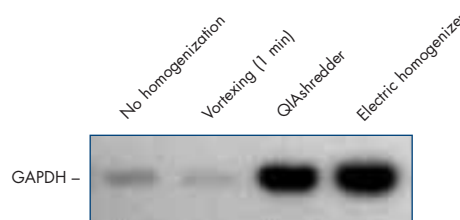
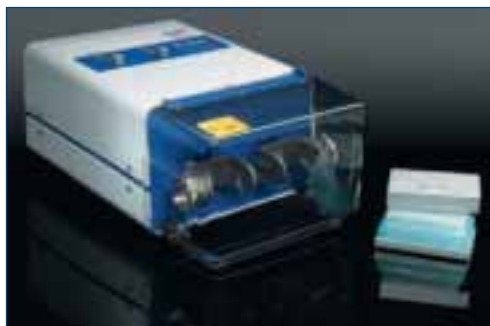


Figure 4 Northern blot of total RNA purified from 5×10^6 HeLa cells using the RNeasy Mini procedure with the indicated homogenization methods. RNA was eluted with $2 \times 40 \mu\text{l}$ water, and $10 \mu\text{l}$ was loaded per lane.

Tissuelyser with
Tissuelyser Adapter Set (96-well)



4.2 High-throughput disruption and homogenization

Tissuelyser system — for high-throughput disruption of a wide range of biological samples

- **Speed** — disruption of up to 192 samples in 2–5 minutes
- **Cross-contamination-free processing** — closed system prevents tube-to-tube carryover
- **Reproducibility** — highly standardized disruption methods
- **Flexibility** — compatible with different sample types and disruption buffers
- **Integrated system** — a key component of the QIAGEN tissue-management system, from sample collection and stabilization to RNA and DNA purification

Efficient Disruption and Homogenization of Animal Tissues

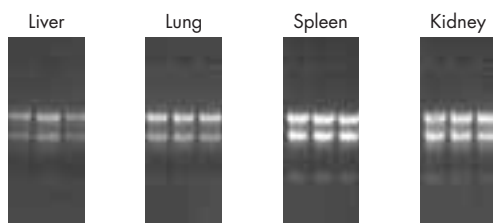


Figure 5 Rat liver, lung, spleen, and kidney tissues were stabilized in RNA/later RNA Stabilization Reagent for 1 year at -20°C and then processed in lysis buffer using the Tissuelyser. Total RNA was purified using the RNeasy Mini Kit and analyzed by formaldehyde agarose electrophoresis.

The Tissuelyser provides rapid and efficient disruption and homogenization of a wide range of samples types, including animal and plant tissues, bacteria, and yeast, at various levels of throughput (Figure 5). Disruption and homogenization using the Tissuelyser gives RNA yields comparable or better than with traditional rotor–stator homogenization methods (Figure 6).

With rotor–stator homogenization, the samples must be processed individually, and the rotor–stator homogenizer must be cleaned after each sample to prevent cross-contamination. In contrast, the Tissuelyser disrupts and homogenizes many samples simultaneously.

The Tissuelyser system is a key component of QIAGEN’s complete solution for tissue management — from sample collection and stabilization to RNA and DNA purification. Over 20 optimized disruption and homogenization protocols integrate the Tissuelyser system with QIAGEN solutions for purification of high-performance nucleic acids from a wide range of sample types.

Comparable or Higher Yields than with Rotor–Stator Homogenization

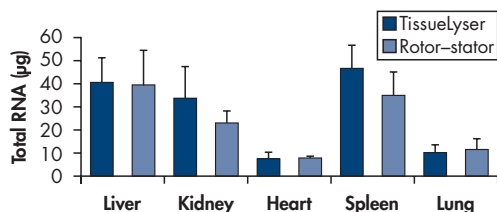


Figure 6 The indicated rat tissues (10 mg each, frozen) were disrupted and homogenized in lysis buffer using the standard Tissuelyser protocol or for 1 minute each using a rotor–stator homogenizer. Total RNA was purified using the RNeasy Mini Kit or, for heart tissue, the RNeasy Fibrous Tissue Mini Kit. RNA yields were determined by absorbance at 260 nm.

The purity of nucleic acids has a critical effect on sensitive downstream applications. Contaminants remaining after RNA purification can reduce the efficiency of downstream applications. For sensitive downstream applications, such as real-time RT-PCR, it is critical that any contaminant is efficiently and reliably removed during RNA purification.

Phenol-guanidine-based methods are commonly used to purify RNA, since phenol is an effective reagent for lysing samples, especially difficult-to-lyse tissues, and since guanidine salts effectively inhibit RNases. However, contaminants remaining in the purified RNA can affect the performance of downstream assays, such as real-time RT-PCR. In addition, yields of RNA can vary between preps, and small RNAs (e.g., 5.8S RNA, 5S RNA, and tRNAs) are also purified (Figure 7). Since small RNAs comprise 15–20% of total RNA, then although the yields of RNA may be high, the actual percentage of mRNA will be low.

RNeasy Advantages over Acid-Phenol-Extraction Methods

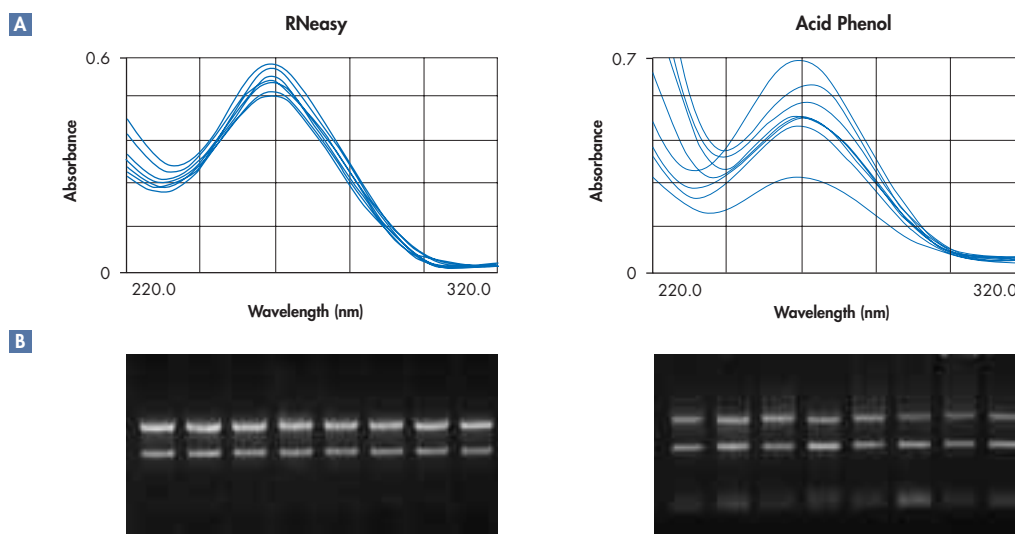


Figure 7 Total RNA was purified from 1×10^6 HeLa cells using the RNeasy Mini Kit or an acid-phenol-extraction method. The RNA purification was performed 8 times with each method. RNA was eluted (**RNeasy**) or resuspended after ethanol precipitation (**Acid Phenol**) in 100 μ l RNase-free water. **A** Aliquots were diluted in 10 mM Tris-Cl, pH 7.5 and analyzed by UV spectrophotometry. **B** 10 μ g of each sample, based on the A_{260} readings in **A**, was analyzed on a formaldehyde agarose gel.

In a real-time RT-PCR study comparing the performance of RNA samples purified from fiber-rich tissue, the C_T values for RNA purified using a standard phenol-guanidine reagent were less reliable than the C_T values for RNA purified using the RNeasy Fibrous Tissue Mini Kit (Figure 8). Contaminants in the phenol-guanidine-purified RNA resulted in a loss of sensitivity and linearity, making quantitative analysis less accurate. For the RNeasy purified RNA, C_T values correlated well with a wide range of template RNA amounts (Figure 8). See page 21 for more information about RNeasy Fibrous Tissue Kits.

Similar observations were made in another real-time RT-PCR study using RNA purified from fatty tissue. When 15 and 30 ng RNA were used as template, samples purified using a standard phenol-guanidine reagent resulted in higher C_T values, indicating some inhibition (Figures 9A and B). For larger amounts of RNA template, it was necessary to use larger volumes of RNA.

This resulted in an increased amount of inhibition (reflected in higher C_T values) when 50 and 100 ng RNA were used as template (Figures 9C and D). RNA purified using the RNeasy Lipid Tissue Mini Kit consistently gave reliable amplification. See page 21 for more information about RNeasy Lipid Tissue Kits.

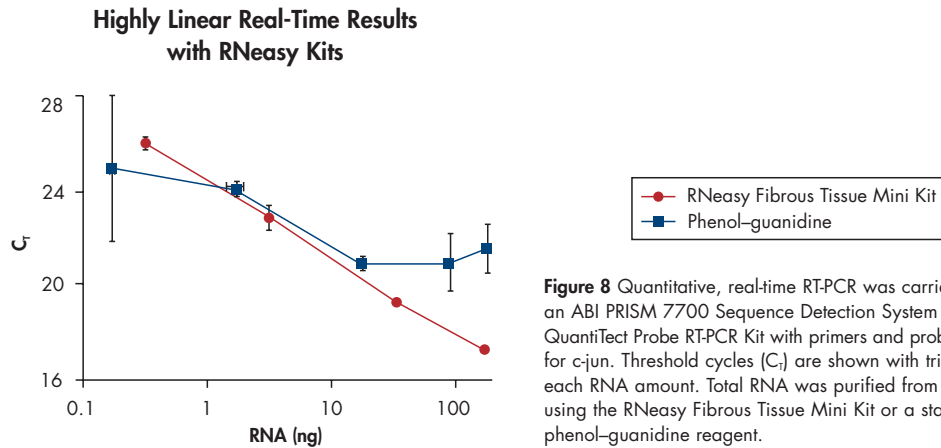


Figure 8 Quantitative, real-time RT-PCR was carried out on an ABI PRISM 7700 Sequence Detection System using the QuantiTect Probe RT-PCR Kit with primers and probes specific for c-jun. Threshold cycles (C_T) are shown with triplicates for each RNA amount. Total RNA was purified from rat muscle using the RNeasy Fibrous Tissue Mini Kit or a standard phenol-guanidine reagent.

Reliable Results with a Wide Range of RNA Amounts Using RNeasy Kits

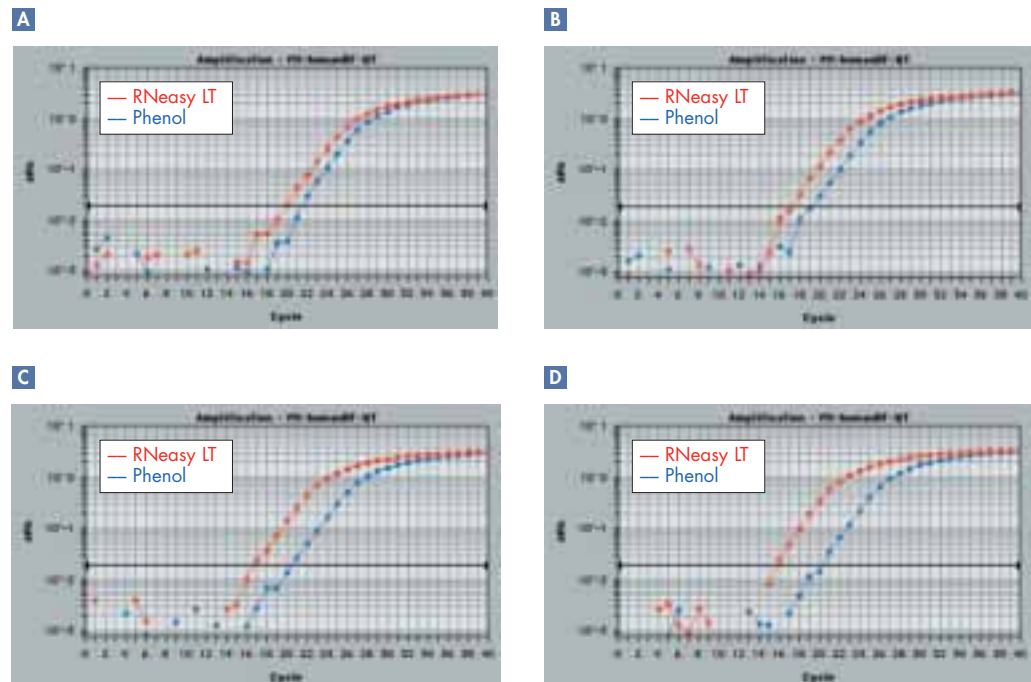


Figure 9 Quantitative, real-time RT-PCR was carried out as described in Figure 8, using the QuantiTect Hs_ACTB Assay, with primers and probe specific for the β -actin transcript. Total RNA was purified from human gland tissue using the RNeasy Lipid Tissue Mini Kit (RNeasy LT) or a phenol-guanidine reagent (Phenol). **A** 15 ng total RNA **B** 30 ng total RNA **C** 50 ng total RNA **D** 100 ng total RNA.

5.1 QIAGEN systems for RNA purification

QIAGEN offers a complete portfolio of products for stabilization and purification of RNA from all types of starting material. Different kits are tailored to suit different RNA purification needs, such as the amount and type of starting material and the number of samples to be processed simultaneously.

QIAGEN RNA purification systems ensure:

- **Excellent recovery of RNA** — from very small to large amounts of starting material; from easy to difficult-to-lyse samples
- **Convenient RNA stabilization and protection in biological samples** — to ensure reliable gene expression and gene-profiling data
- **Ready-to-use, high-quality RNA** — using a rapid procedure
- **Superior performance** — in all downstream applications
- **Highly reproducible RNA purification** — between experiments and between users

With QIAGEN systems for RNA purification, biological samples are disrupted and homogenized in specialized lysis buffers that ensure inactivation of RNases. RNA is then purified, usually using silica-based technology, where RNA selectively binds to silica in the presence of high concentrations of chaotropic salts. The bound RNA is washed several times to remove contaminants such as proteins and metabolites, and then eluted using a low-salt buffer.

With all QIAGEN systems for RNA purification, intact RNA is selectively isolated and efficiently washed, giving high-quality RNA that performs well in all downstream applications, including RT-PCR, quantitative, real-time RT-PCR, array analysis, northern, dot, and slot blotting, and poly A⁺ RNA selection.

RNA samples purified using QIAGEN systems typically give high RNA Integrity Number (RIN) values on the Agilent 2100 bioanalyzer. A RIN value indicates the integrity of an RNA sample, and is more reliable than comparing the ratio of ribosomal bands. This is because the entire sample is considered, including the absence or presence of degradation products. RIN values can also be used to check the variation in quality between RNA preps from the same starting material. The RIN value of an RNA sample strongly depends on the type of starting material from which the RNA was purified. For example, RIN values of RNA purified from cultured cells are usually higher than RIN values of RNA purified from tissue. In a typical experiment using RNeasy technology to purify RNA from cultured cells, RIN values of 9.5 to 10 should be obtained (RIN values range from 1 to 10, with a value of 10 representing the most intact RNA).

The remainder of this section provides a brief overview of the different systems from QIAGEN for RNA purification. Further information can be found in the product profiles and selection guides at the back of this brochure. Alternatively, visit www.qiagen.com, and look under “Products & Services” for “RNA Stabilization and Purification” and “RNA Cleanup & Concentration”.

5.2 Purification of total RNA from small samples

RNeasy Micro Kit — for purification of concentrated total RNA from small amounts of tissue or small numbers of cells

- Reliable RNA purification from small samples, including microdissected tissues, fibrous tissues, and cells
- High-quality total RNA concentrated in only 10 µl for sensitive downstream applications
- Effective removal of genomic DNA with integrated on-column DNase digestion
- Maximal yields of RNA from very small samples, even from single cells

The RNeasy Micro Kit uses a novel technology to purify total RNA from small amounts of cells or tissues, such as laser-microdissected (LMD) tissues, fine-needle aspirates (FNA), and FACS sorted cells. Up to 45 µg RNA can be purified in a volume as low as 10 µl. RNA is reproducibly purified (Figure 10), making it well-suited for sensitive downstream applications, such as array analysis and quantitative, real-time RT-PCR, where the starting material can be as little as one cell (Figure 11).

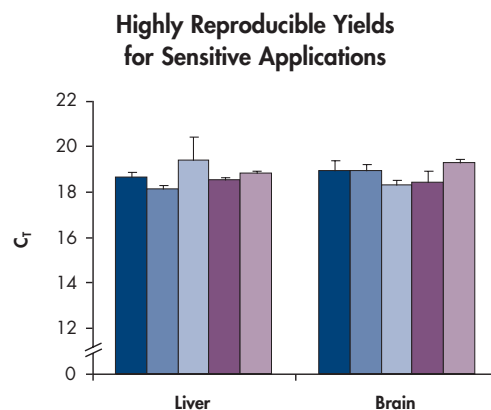


Figure 10 Total RNA was purified by 5 different users in duplicate from 2 mg of the indicated rat tissues, using the RNeasy Micro Kit. Quantitative, real-time RT-PCR was carried out on an ABI™ Sequence Detection System using the QuantiTect Probe RT-PCR Kit, with primers and probes specific for c-jun.

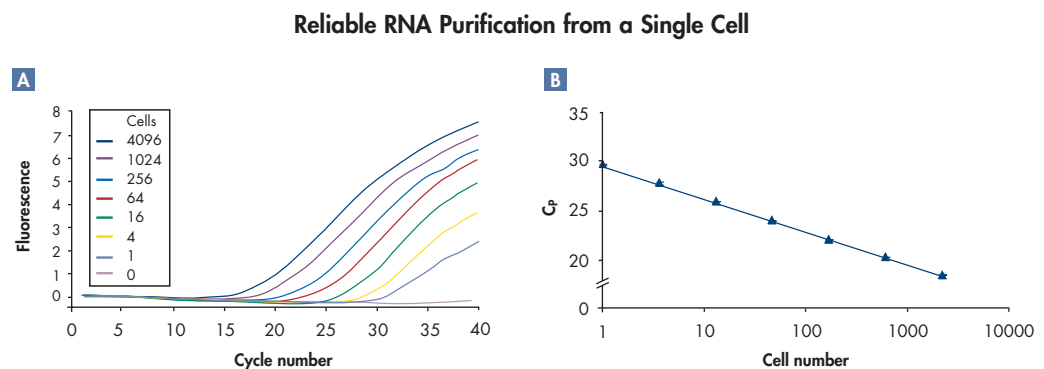


Figure 11 Total RNA was purified from the indicated number of HeLa cells using the RNeasy Micro Kit. **A** Amplification plot. **B** Correlation of crossing point (C_t) values and cell numbers. Real-time RT-PCR was carried out on the LightCycler® System using the QuantiTect Probe RT-PCR Kit, with primers and probes specific for β -actin.

5.3 Purification of total RNA from cells

RNeasy Mini, Midi, and Maxi Kits — for purification of up to 100 µg, 1 mg, or 6 mg total RNA from cultured cells and yeast

- High-quality total RNA in as fast as 20 minutes (mini) or 1 hour or less (midi/maxi)
- No phenol/chloroform extraction, no CsCl gradients, no LiCl or ethanol precipitation
- Excellent recovery of RNA
- Ready-to-use RNA for any downstream application

The kits enable purification of high-quality total RNA from a wide range of cell types (Table 2 and Figure 12), and can also be used to purify total RNA from yeast, bacteria, and standard tissues, such as liver, kidney, and spleen. For RNA purification from small numbers of cells and for high-throughput and/or automated RNA purification from cells, see pages 18 and 28, respectively.

Table 2. Total RNA Yields Obtained with RNeasy Mini, Midi, and Maxi Kits

Cells	RNeasy Mini Kit		RNeasy Midi Kit		RNeasy Maxi Kit	
	No. of cells	Average yield (µg)	No. of cells	Average yield (µg)	No. of cells	Average yield (mg)
LMH	1 × 10 ⁶	12	7 × 10 ⁷	850	5 × 10 ⁸	5.7
HeLa	1 × 10 ⁶	15	7 × 10 ⁷	1000	4 × 10 ⁸	6.0
COS-7	1 × 10 ⁶	35	3 × 10 ⁷	950	1.8 × 10 ⁸	5.8
Lymphocytes (unstimulated)	1 × 10 ⁶	0.5	1 × 10 ⁸	50	5 × 10 ⁸	0.3
<i>S. cerevisiae</i>	1 × 10 ⁷	25	2 × 10 ⁸	450	1 × 10 ⁹	2.4

Amounts can vary due to developmental stage, species, growth conditions used, etc. Since the RNeasy procedure enriches for RNA species >200 nt, RNA yield does not include 5S rRNA, tRNAs, or other low-molecular-weight RNAs.

RT-PCR of RNA from ≥100 Cells

M 10² 5×10² 10³ 5×10³ 10⁴ C- C+ M



Figure 12 RT-PCR of total RNA purified with the RNeasy Mini Kit from the indicated numbers of HeLa cells. 10 µl (1/5) of eluate was digested with RNase-free DNase and reverse transcribed with oligo-dT primer. 2.5 µl (1/20) of the cDNA mix was used in 50 µl PCR. A 452 bp fragment of GAPDH was amplified. C-: negative control; C+: positive control; M: 100 bp ladder.

5.4 Purification of total RNA from tissues

QIAGEN offers a range of kits for purification of total RNA from animal tissues, with each kit optimized for a particular type of tissue sample (see Table 3).

Table 3. RNeasy Kits for RNA Purification from Different Tissue Types

Tissue type	Kit	Purpose of kit
Standard tissues (e.g., liver, kidney, and spleen)	RNeasy Protect Kits	Sample stabilization and total RNA purification
	RNeasy Mini, Midi, and Maxi Kits (see page 19)	Total RNA purification
Fiber-rich tissues (e.g., heart, muscle, and aorta)	RNeasy Fibrous Tissue Kits	Total RNA purification
Fatty tissues (e.g., brain and adipose tissue)	RNeasy Lipid Tissue Kits (also compatible with other types of tissue)	Total RNA purification

For RNA purification from small tissue samples and for high-throughput and/or automated RNA purification from tissues, see pages 18 and 28, respectively.

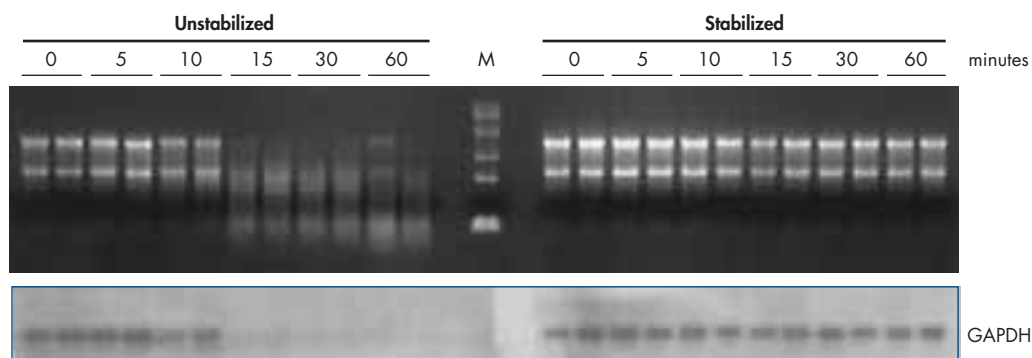
RNeasy Protect Kits — for immediate stabilization of the gene expression profile in animal tissues and subsequent RNA purification

- Immediate sample stabilization and RNA protection ensure reliable gene expression and gene-profiling data
- Convenient and safe processing of samples; no need for liquid nitrogen, dry ice, or hot phenol
- Ready-to-use, high-quality total RNA in minutes
- Excellent recovery of RNA using RNeasy spin-column technology

RNeasy Protect Kits provide a complete solution for stabilizing tissue samples and purifying high-quality total RNA (Figure 13). The supplied RNA_{later} RNA Stabilization Reagent (page 10) ensures immediate stabilization of gene expression profiles when harvesting tissues. RNeasy Protect Mini, Midi, and Maxi Kits are available for purification of up to 100 µg (mini), 1 mg (midi), or 6 mg (maxi) of total RNA per sample.

RNA_{later} Reagent Prevents Degradation of mRNA in Tissues

Figure 13 RNA was purified from fresh rat kidney samples after 0, 5, 10, 15, 30, and 60 minutes, using either standard procedures (**Unstabilized**) or RNeasy Protect Kits (**Stabilized**). The RNA purified was analyzed by agarose gel electrophoresis and expression of GAPDH was examined using northern blot analysis. **M**: markers.



RNeasy Fibrous Tissue Kits — for purification of total RNA from fiber-rich tissues

- Optimized protocols for fibrous tissues
- High yields of total RNA
- High-quality RNA for all downstream applications, such as real-time RT-PCR and array analysis

RNeasy Fibrous Tissue Kits are optimized for purification of high-quality total RNA from fiber-rich tissues (Figure 14), using proteinase K to digest abundant proteins and DNase I to remove trace amounts of genomic DNA. RNeasy Fibrous Tissue Mini and Midi Kits are available for purification of up to 100 µg (mini) and 1 mg (midi) of total RNA per sample. To ensure accurate gene expression analysis, tissue samples should be treated immediately after harvesting with RNA_{later} RNA Stabilization Reagent (page 10).

RNeasy Lipid Tissue Kits — for purification of total RNA from fatty tissues (and from other types of tissue)

- Purification of RNA from larger amounts of fatty tissue
- Optimized lysis conditions for fatty tissues
- High yields of total RNA without phenol contamination
- High-quality RNA for all downstream applications, such as real-time RT-PCR and array analysis

RNeasy Lipid Tissue Kits are optimized for purification of total RNA from fatty tissues, using QIAzol Lysis Reagent to ensure efficient lysis. The purified RNA is free of phenol (Figure 15) and of high quality (Figure 16). RNeasy Lipid Tissue Mini and Midi Kits are available for purification of up to 100 µg (mini) and 1 mg (midi) of total RNA per sample. Starting materials can be up to 100 mg (mini) or 500 mg (midi) of fatty tissue. To ensure accurate gene expression analysis, tissue samples should be treated immediately after harvesting with RNA_{later} RNA Stabilization Reagent (page 10).

Real-Time Gene Expression Analysis in a Variety of Fibrous Tissues

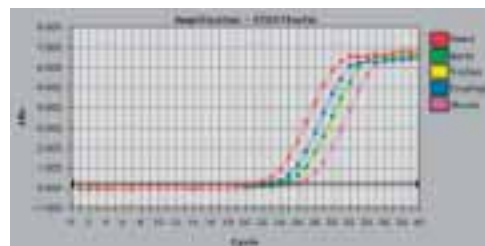


Figure 14 RNA was purified from the indicated rat tissues using the RNeasy Fibrous Tissue Mini Kit. Real-time, quantitative RT-PCR amplification was carried out with 100 ng total RNA using the QuantiTect Probe RT-PCR Kit and primers and probe specific for β -actin.

High Yields of RNA without Phenol Carryover

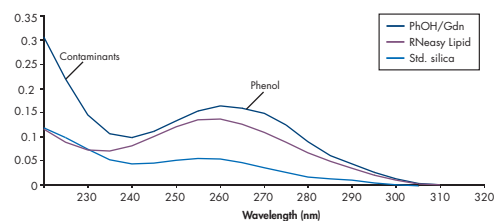


Figure 15 RNA was purified from 10 mg rat brain tissue using the RNeasy Lipid Tissue Mini Kit (**RNeasy Lipid**), a standard silica-gel-membrane procedure (**Std. Silica**), or a phenol-guanidine-based reagent (**PhOH/Gdn**), following suppliers' instructions. Absorbance spectrum shows contaminants and phenol when using the phenol-guanidine-based reagent.

Real-Time Analysis of High-Quality RNA from Fatty Tissues

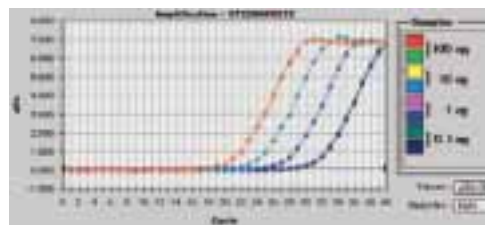


Figure 16 Total RNA was purified from 100 mg rat brain using the RNeasy Lipid Tissue Mini Kit. Real-time, quantitative RT-PCR was carried out using the QuantiTect Probe RT-PCR Kit with the indicated amounts of total RNA (in duplicate) and primers and probe specific for the β -actin gene.

5.5 Purification of total RNA from whole blood

PAXgene Blood RNA system — for blood collection, and RNA stabilization and purification

- Integrated system for collection, stabilization, and purification of intracellular RNA from whole blood
- Immediate stabilization of intracellular RNA, providing in vivo snapshots of gene expression profiles
- RNA stabilization for up to 5 days at 18–22°C
- Standardized sample processing prior to analysis
- No organic extraction or precipitation

The PAXgene Blood RNA system is an integrated and standardized system for collecting and stabilizing whole blood specimens and purifying cellular RNA. Blood samples are collected directly into PAXgene Blood RNA Tubes, which contain a proprietary blend of reagents that enable immediate stabilization of RNA (Figures 17 and 18). RNA is then purified in spin-column format using the PAXgene Blood RNA Kit or in 96-well plate format using the PAXgene 96 Blood RNA Kit.

Stabilization of Gene Expression Using the PAXgene Blood RNA System

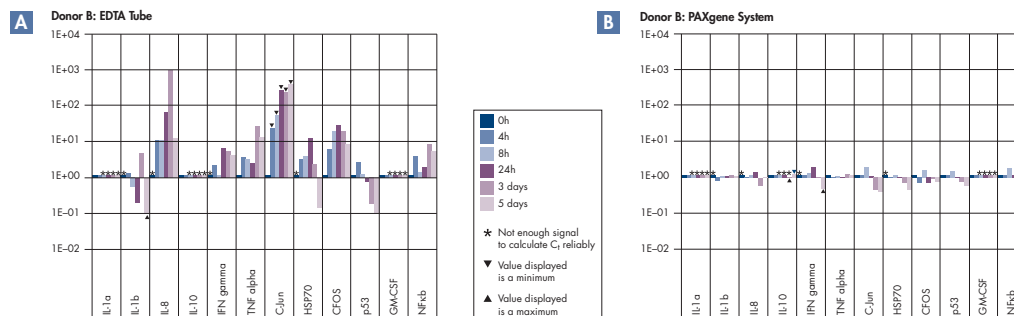


Figure 17 Blood was collected and RNA was purified at the time points shown using either **A** standard methods (collection in EDTA tubes; no stabilization; RNA purification using a guanidine-based method), or **B** the PAXgene Blood RNA system (for RNA stabilization and purification). The graphs show changes in expression of 12 genes after blood collection, measured using real-time RT-PCR.

Detection of Tyrosinase Following Blood Storage

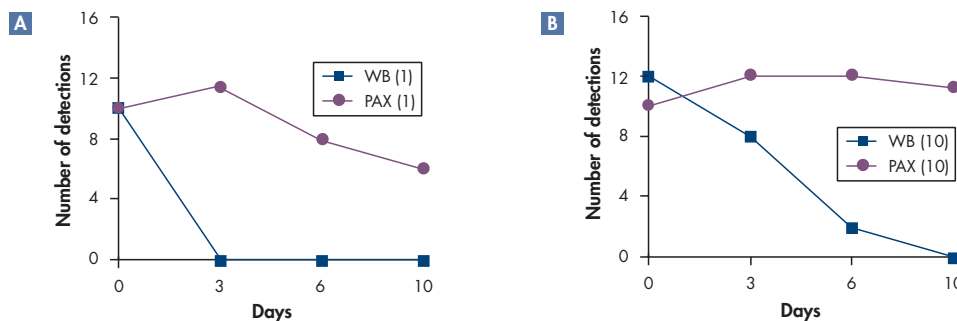


Figure 18 Blood samples were collected from 12 healthy donors into Vacutainer® tubes containing EDTA without stabilization (**WB**) or into PAXgene Blood RNA Tubes (**PAX**). All samples were spiked with melanoma cells. **A** 1 cell per 2.5 ml blood; **B** 10 cells per 2.5 ml blood. RNA was purified using either the PAXgene Blood RNA Kit (**PAX**) or standard methods (**WB**). (R&D data kindly provided by LMO, Munich, Germany.)

QIAamp® RNA Blood Mini Kit — for purification of cellular RNA from fresh whole blood

- Rapid purification of high-quality, ready-to-use total RNA
- No organic extraction or alcohol precipitation
- Consistent, high yields
- Complete removal of contaminants and inhibitors for reliable downstream applications

The QIAamp RNA Blood Mini Kit is designed for purification of cellular RNA from up to 1.5 ml of fresh, human whole blood treated with any common anticoagulant, such as citrate, heparin, or EDTA. The QIAamp procedure completely removes RNases, contaminants, and enzyme inhibitors, yielding high-quality RNA suitable for any downstream application (Figure 19).

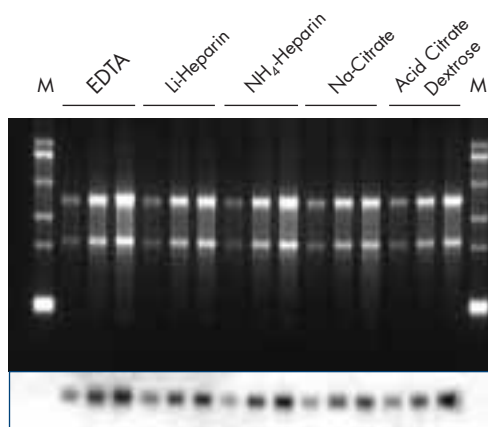
High-Quality RNA for Northern Analysis

Figure 19 Formaldehyde agarose gel and corresponding northern blot (GAPDH-probed) of total RNA purified with the QIAamp RNA Blood Mini Kit from 0.5, 1.0, and 1.5 ml (left to right) of human whole blood from a healthy individual (with indicated anticoagulants). 40 µl of a 60 µl eluate were loaded per lane. **M:** 0.24–9.5 kb RNA ladder.

Detection of Viruses from a Variety of Plant Species by RT-PCR

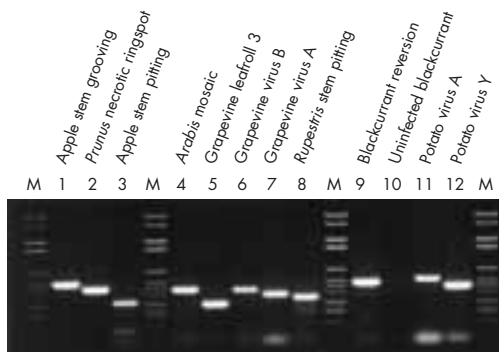


Figure 20 Total RNA was isolated from budwood obtained from infected apple (1), cherry (2), apple (3), and grapevine (4–8), leaf tissue from blackcurrant (9, 10), and dormant tuber tissue from infected potato (11, 12) using a modification of the RNeasy protocol. Viral RNA was amplified using a one-tube RT-PCR. Amplification products were analyzed on a 1.5% agarose gel. **M**: DNA Markers VI, Boehringer Mannheim. (Data kindly provided by D.J. MacKenzie, Centre for Plant Health, Agriculture and Agri-Food Canada and M.A. McLean, Euro Nursery & Vineyard (West) Inc., Sidney, Canada.)

Tissue Specificity of Histone H4 Expression

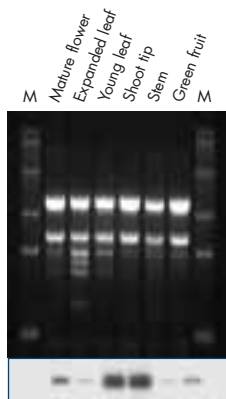


Figure 21 Formaldehyde agarose gel and northern blot of total RNA (7 µg) from indicated tissues of tomato. Blot was hybridized with a ³²P-labeled DNA probe for the tomato histone H4 gene. **M**: 0.24–9.5 kb RNA ladder. (Plant tissues and histone H4 cDNA clone kindly provided by K. Theres, MPI for Breeding Research, Cologne, Germany.)

5.6 Purification of total RNA from plants and fungi

RNeasy Plant Mini Kit — for purification of up to 100 µg total RNA from plants and fungi

- High-quality total RNA in 30 minutes
- No phenol/chloroform extraction, no CsCl gradients, no LiCl or ethanol precipitation
- Excellent recovery of RNA
- Ready-to-use RNA for any downstream application

The RNeasy Plant Mini Kit enables purification of high-quality total RNA from a wide range of plant and fungal samples (Table 4, and Figures 20 and 21). The supplied QIAshredder homogenizers ensure efficient homogenization and filtration of viscous plant and fungal lysates.

Table 4. Selected Samples Processed with the RNeasy Plant Mini Kit

Plants	
<i>Anemone</i> sp.	<i>Oryza sativa</i> (rice)
<i>Arabidopsis thaliana</i> ¹	<i>Pelargonium</i> sp. (geranium)
<i>Begonia</i> sp.	<i>Petroselinum crispum</i> (parsley) ⁷
<i>Beta vulgaris</i> (sugar beet) ²	<i>Pisum sativum</i> (pea)
<i>Chlamydomonas reinhardtii</i> (unicellular alga)	<i>Prunus</i> sp. (cherry) ⁶
<i>Chrysanthemum</i>	<i>Ranunculus</i> sp.
<i>Clarkia</i> spp. ³	<i>Ribes nigrum</i> (black currant)
<i>Daucus carota</i> (carrot) ⁴	<i>Riccia fluitans</i> (liverwort)
<i>Diascia</i> sp.	<i>Sinapis arvensis</i> (mustard)
<i>Dendranthema</i> sp.	<i>Solanum tuberosum</i> (potato) ⁸
<i>Euglena gracilis</i> (unicellular alga)	<i>Spinacia oleracea</i> (spinach)
<i>Funaria hygrometrica</i> (moss)	<i>Surfinia</i> sp.
<i>Hordeum vulgare</i> (barley)	<i>Triticum aestivum</i> (wheat)
<i>Lycopersicon esculentum</i> (tomato) ⁵	<i>Vitis</i> sp. (grapevine) ⁶
<i>Malus</i> sp. (apple) ⁶	<i>Zea mays</i> (maize)
<i>Nicotiana tabacum</i> (tobacco)	
Filamentous fungi	
<i>Acremonium crysogenum</i> ⁹	<i>Fusarium avenaceum</i> ⁹

Young leaves or needles, except as indicated: ¹Flowers, buds, siliques, roots; ²roots; ³petals, stamen, stigma, sepals, style; ⁴cultured cells; ⁵mature flower, expanded leaves, shoot tip, stem, green fruit; ⁶budwood; ⁷seedlings; ⁸dormant tuber; ⁹mycelium.

5.7 Purification of total RNA from bacteria

RNeasy Protect Bacteria Kits — for in vivo stabilization of the gene expression profile in bacteria and subsequent RNA purification

- Immediate stabilization of the gene expression profile prior to cell harvest and RNA purification
- Convenient procedure; stabilization reagent is added directly to bacterial cultures
- Ready-to-use, high-quality total RNA in minutes
- Improved sensitivity in downstream applications

RNeasy Protect Bacteria Kits provide a complete solution for stabilization and purification of high-quality total RNA from bacteria (Figure 22). The supplied RNAprotect Bacteria Reagent (page 11) ensures in vivo stabilization of gene expression profiles. RNA can be purified from Gram-positive and Gram-negative bacteria, and from bacteria grown in minimal or complex medium. RNeasy Protect Bacteria Mini and Midi Kits are available for stabilization and purification of up to 100 µg (mini) and 1 mg (midi) of total RNA per sample.

GeneChip® Analysis Shows Accurate Gene-Expression Profiles with RNAprotect Stabilization

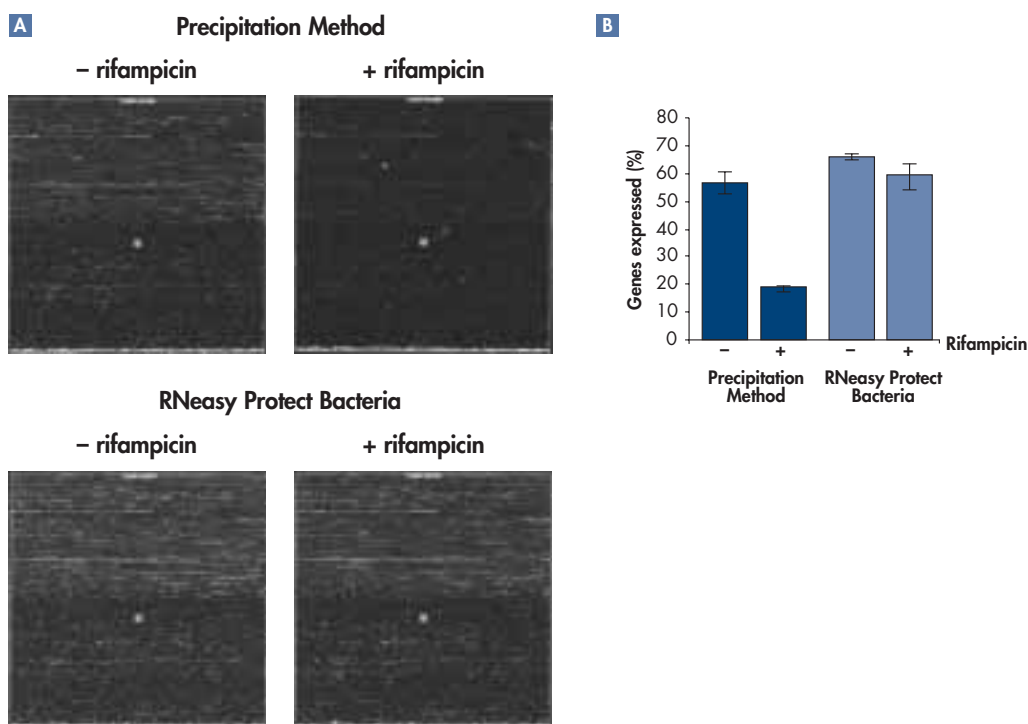


Figure 22 Total RNA was purified from RNAprotect stabilized *E. coli* cultures using the RNeasy Protect Bacteria Kit (**RNeasy Protect Bacteria**) or from unstabilized cultures using a precipitation method (**Precipitation Method**). To distinguish between gene expression under defined culture conditions and effects of artifactual gene induction during harvesting and RNA purification, the RNA polymerase inhibitor rifampicin was added to half of the culture prior to RNA purification. Differences in transcript levels with and without rifampicin therefore generally reflect the degree of RNA degradation. **A** GeneChip analysis of *E. coli* microarrays was carried out according to standard Affymetrix protocols. **B** The percentage of genes expressed was estimated as the number of different transcripts determined present by GeneChip analysis divided by the total number of transcripts represented on the microarray. (Data from a collaborative study with Affymetrix.)

5.8 Purification of small RNA or both total RNA and genomic DNA

QIAGEN RNA/DNA Kits — for purification of total RNA, parallel purification of RNA and genomic DNA, and purification of low-molecular-weight RNA

- Ultrapure RNA and DNA from the same sample, even from small amounts of precious biopsy tissue or small numbers of cells
- Flexibility for parallel RNA and DNA purification, low-molecular-weight RNA purification, total RNA purification, or RNA cleanup
- No phenol/chloroform extraction or CsCl ultracentrifugation

QIAGEN RNA/DNA Kits can be used for RNA and DNA purification from animal cells and tissues, yeast, and bacteria as well as for purification of in vitro transcripts and cleanup of RNA. Depending on the procedure followed, low-molecular-weight RNA (e.g., tRNA and 5S rRNA), total RNA, or both total RNA and genomic DNA are purified using QIAGEN-tips (Figures 23 and 24). Kits are available in mini, midi, or maxi format for purification of up to 40, 200, 1000 µg RNA and/or up to 20, 100, 500 µg DNA.

Parallel Purification of RNA and DNA

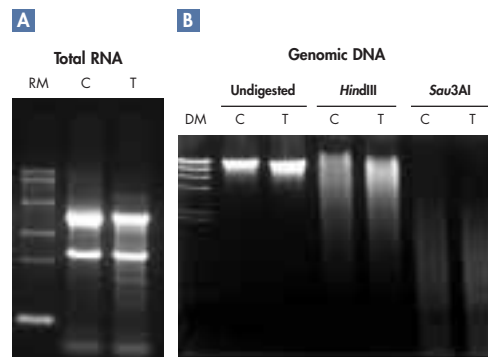


Figure 23 Parallel purification of total RNA and genomic DNA using the QIAGEN RNA/DNA Kit. **A** Formaldehyde agarose gel (1.2%) of total RNA and **B** TBE agarose gel (0.8%) of genomic DNA purified from 1×10^7 chicken liver cells (**C**) or 50 mg mouse liver tissue (**T**) using QIAGEN-tip 100. Yields from (**C**) and (**T**) were 135 and 210 µg RNA, and 62 and 79 µg DNA, respectively. Total RNA (12.5 µg) and genomic DNA, undigested (2 µg) or digested (4 µg) as indicated, were loaded. **RM**: 0.24–9.5 kb RNA markers; **DM**: lambda-HindIII.

Purification of Small RNAs

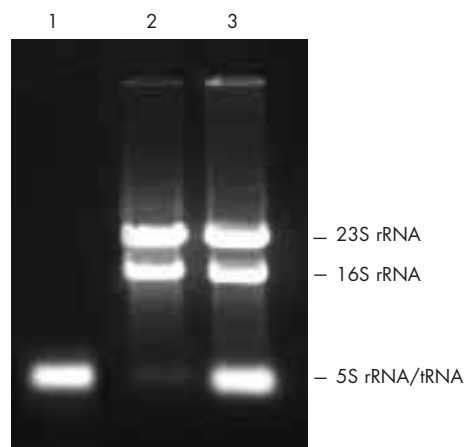


Figure 24 Purification of small RNAs (5S rRNA and tRNA) using the QIAGEN RNA/DNA Kit. Lysates were prepared from 4×10^9 *E. coli* (HB101) cells and loaded onto a QIAGEN-tip 100. **1**: LMW fraction (5S rRNA and tRNAs) eluted with Buffer QRW2; **2**: remaining RNA molecules eluted using prewarmed (45°C) Buffer QRU[®]; **3**: RNA obtained using the standard QIAGEN RNA/DNA protocol. 10 µl of RNA (of a total eluate of 100 µl) was loaded in each lane.

5.9 Purification of poly A⁺ mRNA

Oligotex® Kits — for purification of poly A⁺ mRNA from total RNA, cells, or tissues, and for cleanup of in vitro transcripts

- High recovery of pure mRNA in as little as 30 minutes
- No oligo-dT cellulose or ethanol precipitation
- Flexibility for use with widely varying amounts of starting RNA

Oligotex mRNA Kits allow purification of pure poly A⁺ mRNA from total RNA preps in less than 30 minutes with >90% recovery, and can also be used to clean up in vitro transcripts. Oligotex Direct mRNA Kits allow purification of pure poly A⁺ mRNA directly from cells or tissues in as little as 1 hour with >90% recovery (Figure 25). For automated purification of mRNA from cultured cells or blood cells, see page 28.

5.10 Cleanup and concentration of RNA

RNeasy MinElute® Cleanup Kit — for RNA cleanup and concentration with small elution volumes

- Efficient cleanup of enzymatic reactions, removing RNases, nucleotides, and other impurities
- Concentration of small amounts of RNA to only 10 µl for sensitive applications
- Cleanup of RNA purified by different methods, including crude preps
- High-quality RNA in less than 15 minutes, with no time-consuming precipitation or vacuum centrifugation

The RNeasy MinElute Cleanup Kit enables purification and concentration of RNA from enzymatic reactions (e.g., in vitro transcription, DNase digestion, and RNA labeling), desalting of RNA samples (e.g., after phenol extraction and ethanol precipitation), and concentration of RNA (e.g., after purification using the RNeasy Mini Kit or PAXgene Blood RNA Kit). RNA ranging from 45 µg down to picogram amounts, corresponding to less than one cell, can be concentrated down to 10 µl, suitable for sensitive downstream applications (Figure 26).

Other QIAGEN kits also provide the option of RNA cleanup. These include RNeasy Micro, Mini, Midi, and Maxi Kits (pages 18 and 19), RNeasy 96 Kits (page 32), Oligotex mRNA Kits (page 27), and QIAGEN RNA/DNA Kits (page 26).

Purification of mRNA from Total RNA

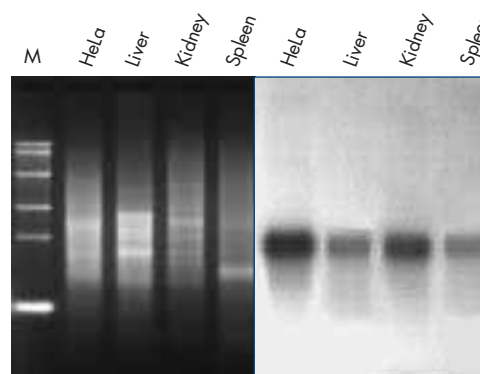


Figure 25 Formaldehyde agarose gel and northern blot (GAPDH-probed) of poly A⁺ mRNA purified from total RNA from various samples using Oligotex Kits. **M:** markers.

Concentration of Picogram Amounts of RNA

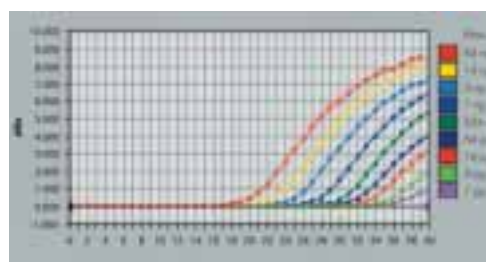


Figure 26 The indicated amounts of total RNA from HeLa cells were concentrated using the RNeasy MinElute Cleanup Kit, and 5 µl of the 12 µl eluate was used for each reaction. Real-time RT-PCR was carried out on an ABI Sequence Detection System using the QuantiTect Probe RT-PCR Kit, with primers and probes specific for β-actin.

5.11 High-throughput and/or automated purification of RNA

For animal cells and tissues, QIAGEN offers kits for high-throughput RNA purification as well as kits for automated RNA purification at a range of throughputs (see Table 5).

Table 5. Manual and Automated Solutions for RNA Purification

Sample type	RNA purified	Samples per run	Robotic workstation required	Kit
Animal cells	Total RNA	1–6	BioRobot® EZ1	EZ1 RNA Cell Mini Kit with EZ1 RNA Card
		6–48	BioRobot M48	MagAttract® RNA Cell Mini M48 Kit
		Up to 96 or 192	None*	RNeasy 96 Kit
		Up to 96 or 192	BioRobot Gene Expression	RNeasy 96 BioRobot 8000 Kit
Animal cells and whole blood	mRNA	6–48	BioRobot M48	MagAttract Direct mRNA M48 Kit
Standard animal tissues	Total RNA	1–6	BioRobot EZ1	EZ1 RNA Tissue Mini Kit with EZ1 RNA Card
		6–48	BioRobot M48	MagAttract RNA Tissue Mini M48 Kit
All types of animal tissue	Total RNA	1–6	BioRobot EZ1	EZ1 RNA Universal Tissue Kit† with EZ1 RNA Universal Tissue Card
		6–48	BioRobot M48	MagAttract RNA Universal Tissue M48 Kit†
		Up to 96 or 192	None‡	RNeasy 96 Universal Tissue Kit
		Up to 96 or 192	BioRobot Gene Expression	RNeasy 96 Universal Tissue 8000 Kit

* Samples are manually processed using the QIAvac 96 vacuum manifold, the QIAGEN 96-Well-Plate Centrifugation system, or a combination of both.

† RNA purification from cultured cells and white blood cells is also possible.

‡ Samples are manually processed using the QIAGEN 96-Well-Plate Centrifugation system and, optionally, the QIAvac 96 vacuum manifold.

BioRobot EZ1 and EZ1 Kits and Cards — for easy, automated purification of RNA from 1–6 cell or tissue samples

- High-quality total RNA, rapidly purified from cells and tissues
- Affordable, slimline workstation, ideal for high priority samples
- Ease of operation
- Safe and contamination-free setup
- Highly reproducible RNA purification

The BioRobot EZ1 is a compact workstation that provides easy automated purification of total RNA from 1–6 samples per run. The user simply inserts a pre-programmed EZ1 Card and then follows the instructions on the LCD on the workstation to load samples and the components of an EZ1 Kit. These include pre-filled reagent cartridges containing reagents for the purification procedure. After loading, the workstation works without interruption to provide pure RNA in under an hour. A range of EZ1 Kits and EZ1 Cards are available for reproducible purification of high-quality RNA from cells and tissues (Figures 27 and 28).

BioRobot EZ1



Consistent Highly Pure RNA

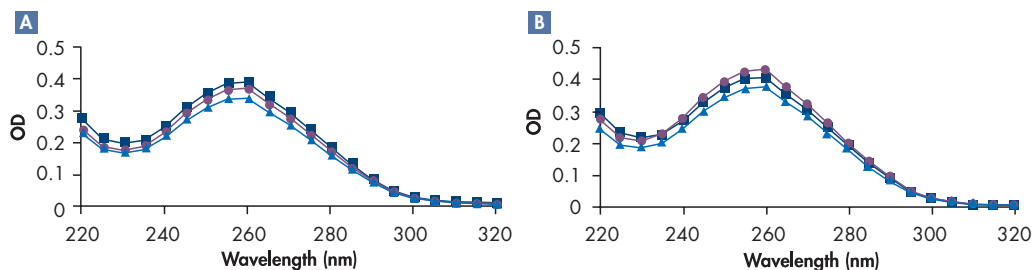


Figure 27 RNA was purified from 2–3 replicate tissue samples from each of 4 types of rat tissue. Purified total RNA was examined by scanning spectrophotometry. **A** Kidney, 10 mg; **B** Pancreas, 5 mg.

Efficient Labeling of Target for Microarray Analysis

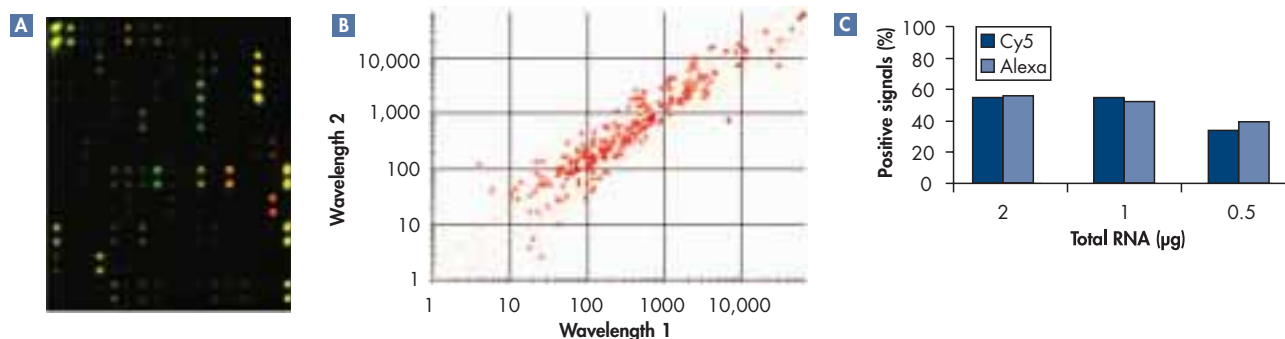


Figure 28 Total RNA was purified from HeLa cells using the BioRobot EZ1 system. cDNA was synthesized from total RNA and simultaneously labeled with both Alexa Fluor® 532 and Cy5 fluorophores using the QIAGEN LabelStar™ Array Kit. Labeled cDNA was hybridized to a SensiChip™ DNA Array Bar containing stress- and aging-specific capture probes. **A** Microarray image from the SensiChip DNA Array Reader following hybridization with 1 µg cDNA. Image was acquired using a 5 second exposure. **B** Plot showing that intensities of signal correlate well following hybridization with 1 µg cDNA, independent of the fluorophore type. **C** A comparison of numbers of positive (signal-to-noise ratio >3) signals detected (5 second exposure) following hybridization with varying amounts of total RNA.

BioRobot M48


BioRobot M48 and MagAttract M48 Kits — for flexible, fully automated RNA purification from 6–48 cell or tissue samples

- High-quality total RNA, purified from cells and tissues
- High-quality mRNA, purified from cultured cells and whole blood
- Easy optimization of RNA yield and concentration
- Highly reproducible RNA purification

The BioRobot M48 workstation provides flexible and cross-contamination-free automated purification of RNA from 6–48 samples, in increments of 6, per run. The user-friendly QIAsoft M Operating System software provides the user with a step-by-step guide to loading the workstation with samples and the reagents from a MagAttract M48 Kit. The software also allows the user to adjust the input sample volume and the output RNA volume, enabling purification of RNA at a volume and concentration appropriate for the intended downstream application. A range of MagAttract M48 Kits are available for reproducible purification of high-quality RNA from cells, tissues, and whole blood (Figures 29 and 30).

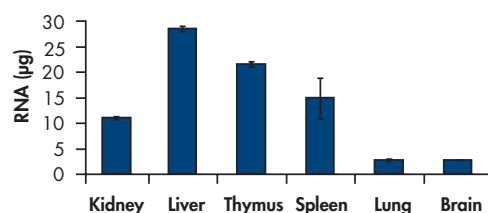
Purification of RNA from Various Tissues


Figure 29 RNA was purified from duplicate samples of kidney (10 mg), liver (10 mg), thymus (10 mg), spleen (5 mg), lung (10 mg), and brain (10 mg) using the BioRobot M48 workstation and the MagAttract RNA Tissue Mini M48 Kit.

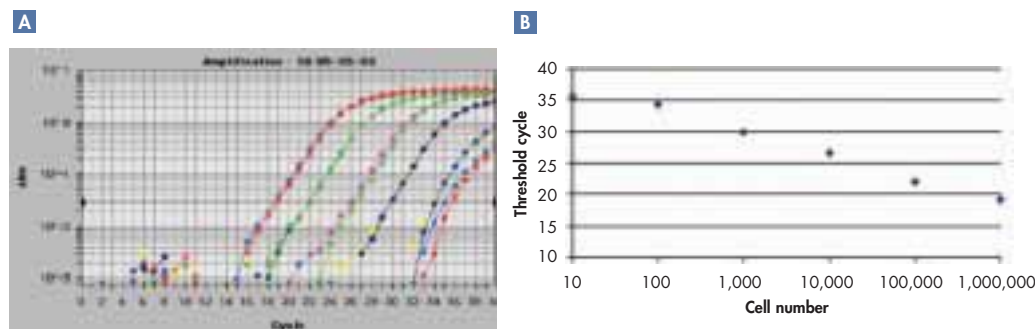
Linear C_T Values Over A Large Dynamic Range


Figure 30 Ten-fold dilution series were performed on lysates of 10^6 HeLa cells to produce 200 μ l aliquots equivalent to 10^1 – 10^6 cells. RNA was purified from dilutions of the lysate using the BioRobot M48 workstation and the MagAttract RNA Cell Mini M48 Kit. Purified RNA was eluted in 200 μ l elution buffer and duplicate aliquots of 5 μ l were used in two 25 μ l real-time RT-PCRs of human c-myc mRNA using the QuantiTect Probe RT-PCR Kit. **A** ABI PRISM 7700 real-time amplification plots. **B** Plot of C_T values and cell number equivalents.

BioRobot Gene Expression — for walkaway RNA purification and RT-PCR setup from cell culture and tissue samples in 96-well format

- Automated purification of total RNA from up to 192 samples
- Accurate real-time analysis, with C_T CVs of <3%
- Highly repeatable RNA yields, with OD CVs <10%
- Comprehensive support, including installation, performance testing, and on-site software training



The BioRobot Gene Expression workstation with the Real-Time RT-PCR Specialist Pack provides a complete walkaway solution for RNA purification and reaction setup for quantitative, real-time RT-PCR. The workstation can either purify RNA from up to 192 samples in one run, or it can purify RNA and perform RT-PCR setup for up to 96 samples in one run. High-throughput purification of total RNA from cells and tissues is provided by the RNeasy 96 BioRobot 8000 Kit and the RNeasy 96 Universal Tissue 8000 Kit, respectively (pages 32 and 33). Disposable filter-tips are used to ensure cross-contamination-free processing of samples (Figure 31).

Cross-Contamination-Free Processing for the Most Sensitive Applications

	1	2	3	4	5	6	7	8	9	10	11	12
A	18	X	18	X	19	X	18	X	18	X	18	X
B	X	18	X	18	X	18	X	18	X	18	X	18
C	18	X	19	X	19	X	19	X	19	X	19	X
D	X	19	X	19	X	19	X	19	X	19	X	18
E	19	X	19	X	19	X	19	X	19	X	18	X
F	X	18	X	19	X	19	X	19	X	18	X	18
G	18	X	18	X	18	X	18	X	18	X	19	X
H	X	18	X	18	X	18	S	S	S	S	S	S

Figure 31 Alternating wells of 96-well cell culture plates contained either 5 x 10⁵ HeLa S3 cells or water (blue). RNA purification was carried out for all wells using the RNeasy 96 BioRobot 8000 procedure. Total RNA was eluted in 100 µl RNase-free water, and 5 µl was used for quantitative, real-time RT-PCR on the ABI Sequence Detection System using the QuantiTect Probe RT-PCR Kit with primers and dual-labeled probe specific for the β-actin transcript. C_T values are indicated. **X**: No signal was detected after 40 PCR cycles. **S**: Standards (not included in the analysis).

RNeasy 96 Kit and RNeasy 96 BioRobot 8000 Kit — for high-throughput manual or automated RNA minipreps from cells

- High-throughput RNA purification
- Fast and convenient sample processing
- No organic extraction or precipitation
- Reproducible yields from 10 to 500,000 cells
- High-quality RNA for any application

The RNeasy 96 Kit and the RNeasy 96 BioRobot 8000 Kit provide purification of total RNA from up to 96 or 192 cultured cell samples in manual and automated formats, respectively. The purified RNA is of high quality and provides reproducible results in downstream applications (Figures 32 and 33).

High-Quality RNA for Sensitive Analysis of a Low-Copy Transcript

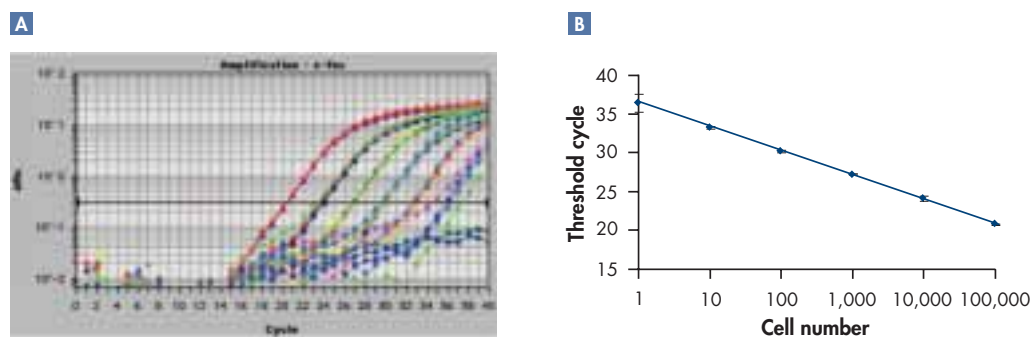


Figure 32 RNA was purified from 1 to 1×10^5 HeLa cells using the RNeasy 96 BioRobot 8000 procedure. Total RNA was eluted in 100 μ l RNase-free water, and 5 μ l was used for RT-PCR. Quantitative, real-time, one-step RT-PCR analysis was carried out on an ABI Sequence Detection System using the QuantiTect Probe RT-PCR Kit with primers and probe specific for the low-copy *c-fos* transcript. **A** Amplification plot **B** C_t values. Error bars represent standard deviation from 4 different samples for each cell number.

High-Throughput Purification and Reaction Setup on a Single Workstation

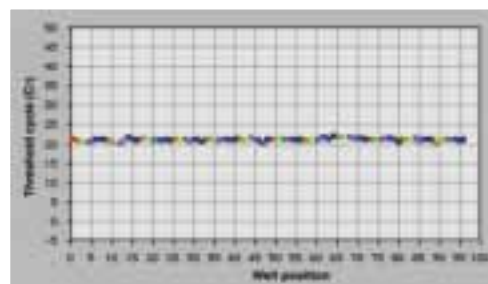


Figure 33 RNA was purified from 96 aliquots (5×10^4 cells each) of a HeLa S3 cell culture using the RNeasy 96 BioRobot 8000 procedure. Quantitative, real-time, one-step RT-PCR was set up in the same protocol on the BioRobot workstation, using the QuantiTect Probe RT-PCR Kit with primers and dual-labeled probe specific for the low-copy *c-myc* transcript. Threshold cycles (C_t) are shown for all 96 samples. The mean C_t was 21.34 ± 0.34 (mean \pm standard deviation), representing a CV of 1.6%.

RNeasy 96 Universal Tissue Kit and RNeasy 96 Universal Tissue 8000 Kit — for high-throughput manual or automated RNA purification from any type of animal tissue

- High yields of total RNA from all types of tissue in 96-well format
- Integration of QIAzol lysis and high-throughput RNeasy purification in an easy-to-follow protocol
- Pure, high-performance RNA without phenol contamination
- High-quality RNA for all downstream applications, such as real-time RT-PCR and array analysis

The RNeasy 96 Universal Tissue Kit and the RNeasy 96 Universal Tissue 8000 Kit provide purification of total RNA from up to 96 or 192 tissue samples in manual and automated formats, respectively. Any type of tissue can be processed, including difficult-to-lyse fibrous and fatty tissues. The kits are integrated with the TissueLyser (page 14), which enables efficient disruption of samples. The purified RNA is free of phenol and of high quality (Figure 34), and provides reproducible results in downstream applications (Figure 35).

High-Quality RNA from Any Tissue Sample

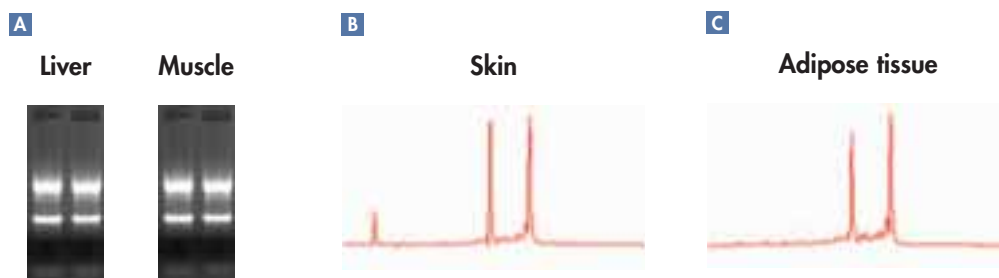


Figure 34 Total RNA was purified from the indicated rat tissues using the RNeasy 96 Universal Tissue Kit and analyzed by formaldehyde agarose gel electrophoresis or on the Agilent 2100 bioanalyzer. Starting materials were **A** 25 mg RNAlater stabilized liver and muscle, **B** 50 mg flash-frozen skin, and **C** 100 mg flash-frozen adipose tissue.

Real-Time Analysis of High-Quality RNA from Rat Brain

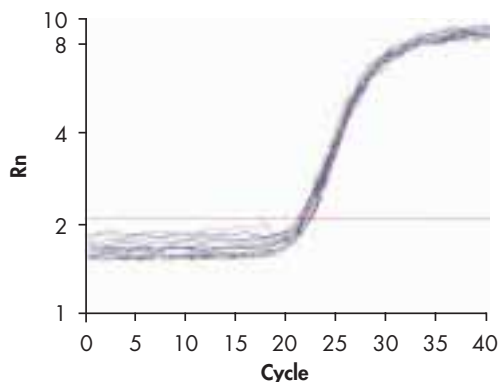


Figure 35 Total RNA was purified from 50 mg samples of rat brain using the RNeasy 96 Universal Tissue Kit. Twenty-four purified samples, with an average of 90 ng RNA per reaction, were analyzed by real-time RT-PCR on the ABI PRISM 7900HT Sequence Detection System, using the QuantiTect Probe RT-PCR Kit with primers and probe specific for the c-jun gene.

5.12 DNase digestion during RNA purification

RNase-Free DNase Set — for DNase digestion during RNA purification

- Guaranteed RNase-free
- Stable, lyophilized enzyme
- Convenient, on-membrane treatment and subsequent DNase removal
- Optimized for use with QIAGEN and PreAnalytiX™ spin-column and 96-well RNA purification procedures

The RNase-Free DNase Set is quality-controlled and optimized for use with RNeasy, RNeasy Protect, RNeasy 96, QIAamp RNA Blood, EZ1 RNA Universal Tissue, and PAXgene Blood RNA Kits.

Generally, DNase digestion is not required for RNA purified with QIAGEN and PreAnalytiX silica-gel-membrane technologies since the majority of the DNA is removed without DNase treatment. However, more complete DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA.

Product	Contents	Cat. no.
Sample collection and stabilization		
RNA ^{later} RNA Stabilization Reagent (50 ml)*	50 ml reagent for stabilizing RNA in 25 x 200 mg tissue samples	76104
RNA ^{later} TissueProtect Tubes (50 x 1.5 ml)	50 screw-top tubes, each containing 1.5 ml reagent for stabilizing 150 mg tissue	76154
RNA ^{later} TissueProtect Tubes (20 x 5 ml)	20 screw-top tubes, each containing 5 ml reagent for stabilizing 500 mg tissue	76163
RNAprotect Bacteria Reagent	2 x 100 ml reagent for stabilizing RNA in bacteria	76506
Sample disruption and homogenization		
QIAshredder (50)*	50 QIAshredder spin columns and caps	79654
TissueLyser [†]	Universal laboratory mixer-mill disruptor	Inquire
TissueLyser Adapter Set 2 x 24 [†]	2 sets of Adapter Plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser	69982
TissueLyser Adapter Set 2 x 96 [†]	2 sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser	69984
Purification of total RNA from small samples		
RNeasy Micro Kit (50)	50 RNeasy MinElute spin columns, Collection Tubes, DNase I, Carrier RNA, Reagents and Buffers	74004
Purification of total RNA from cells		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes, Reagents and Buffers	74104
RNeasy Midi Kit (10)* [‡]	10 RNeasy Midi Spin Columns, Collection Tubes, Reagents and Buffers	75142
RNeasy Maxi Kit (12) [‡]	12 RNeasy Maxi Spin Columns, Collection Tubes, Reagents and Buffers	75162
Purification of total RNA from tissues		
RNeasy Protect Mini Kit (50)*	RNA ^{later} RNA Stabilization Reagent, 50 RNeasy Mini Spin Columns, Collection Tubes, Reagents and Buffers	74124
RNeasy Protect Midi Kit (10)* [‡]	RNA ^{later} RNA Stabilization Reagent, 10 RNeasy Midi Spin Columns, Collection Tubes, Reagents and Buffers	75152
RNeasy Protect Maxi Kit (12) [‡]	RNA ^{later} RNA Stabilization Reagent, 12 RNeasy Maxi Spin Columns, Collection Tubes, Reagents and Buffers	75182
RNeasy Fibrous Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes, Proteinase K, DNase I, Reagents and Buffers	74704
RNeasy Fibrous Tissue Midi Kit (10) [‡]	10 RNeasy Midi Spin Columns, Collection Tubes, Proteinase K, DNase I, Reagents and Buffers	75742

* Larger kit sizes available; for details, visit www.qiagen.com.

[†] Visit www.qiagen.com/goto/bro/accessories to view the full range of TissueLyser accessories, including beads, bead dispensors, and tubes.

[‡] Requires use of a centrifuge capable of attaining 3000–5000 x g equipped with a swing-out rotor for 15 ml (midi) or 50 ml (maxi) centrifuge tubes.

Product	Contents	Cat. no.
RNeasy Lipid Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes, QIAzol Lysis Reagent, Reagents and Buffers	74804
RNeasy Lipid Tissue Midi Kit (10)*	10 RNeasy Midi Spin Columns, Collection Tubes, QIAzol Lysis Reagent, Reagents and Buffers	75842
QIAzol Lysis Reagent (200 ml)	200 ml reagent for lysing fatty tissues	79306
Purification of total RNA from whole blood		
PAXgene Blood RNA Tubes (100)	100 PAXgene Blood RNA Tubes	Inquire
PAXgene Blood RNA Kit (50)	50 PAXgene RNA Spin Columns, Buffers, Proteinase K, Processing Tubes	762134
PAXgene 96 Blood RNA Kit (4)	4 PAXgene 96 RNA Plates, 4 PAXgene 96 Filter Plates, Buffers, Proteinase K, DNase I, Blocks and Tubes	762331
PAXgene Blood RNA Validation Kit (10)	10 PAXgene Blood RNA Tubes, 10 PAXgene RNA Spin Columns, Buffers, Proteinase K, Processing Tubes	762132
QIAamp RNA Blood Mini Kit (50)	50 QIAamp Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes, Reagents and Buffers	52304
Purification of total RNA from plants and fungi		
RNeasy Plant Mini Kit (20)†	20 RNeasy Mini Spin Columns, 20 QIAshredder Spin Columns, Collection Tubes, Reagents and Buffers	74903
Purification of total RNA from bacteria		
RNeasy Protect Bacteria Mini Kit (50)	RNeasy Mini Kit (50) and RNAprotect Bacteria Reagent	74524
RNeasy Protect Bacteria Midi Kit (10)*	RNeasy Midi Kit (10) and RNAprotect Bacteria Reagent	75552
Purification of poly A⁺ mRNA‡		
Oligotex mRNA Mini Kit (12)	200 µl Oligotex Suspension, Small Spin Columns, Collection Tubes, Reagents and Buffers	70022
Oligotex mRNA Midi Kit (12)	700 µl Oligotex Suspension, Small Spin Columns, Collection Tubes, Reagents and Buffers	70042
Oligotex mRNA Maxi Kit (6)	700 µl Oligotex Suspension, Large Spin Columns, Collection Tubes, Reagents and Buffers	70061
Oligotex Direct mRNA Micro Kit (12)	250 µl Oligotex Suspension, Small Spin Columns, Collection Tubes, Reagents and Buffers	72012

* Requires use of a centrifuge capable of attaining 3000–5000 × g equipped with a swing-out rotor for 15 ml (midi) or 50 ml (maxi) centrifuge tubes.

† Larger kit sizes available; for details, visit www.qiagen.com.

‡ Oligotex Kits are not available in Japan.

Product	Contents	Cat. no.
Oligotex Direct mRNA Mini Kit (12)	420 µl Oligotex Suspension, Small Spin Columns, Collection Tubes, Reagents and Buffers	72022
Oligotex Direct mRNA Midi/Maxi Kit (6/2)	1 ml Oligotex Suspension, Large Spin Columns, Collection Tubes, Reagents and Buffers	72041
Oligotex Suspension (0.5 ml)	0.5 ml suspension for purifying mRNA from up to 8 mg of total RNA	79000
Oligotex Suspension (2.5 ml)	2.5 ml suspension for purifying mRNA from up to 40 mg of total RNA	79002
Small Spin Columns (24)	24 small spin columns for Oligotex spin procedures	79523
Purification of small RNA or both total RNA and genomic DNA		
QIAGEN RNA/DNA Mini Kit (25)	25 QIAGEN-tip 20, Reagents and Buffers	14123
QIAGEN RNA/DNA Midi Kit (10)	10 QIAGEN-tip 100, Reagents and Buffers	14142
QIAGEN RNA/DNA Maxi Kit (10)	10 QIAGEN-tip 500, Reagents and Buffers	14162
Cleanup and concentration of RNA		
RNeasy MinElute Cleanup Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes, Reagents and Buffers	74204
High-throughput manual purification of RNA from up to 96 or 192 samples		
RNeasy 96 Kit (4)*	4 RNeasy 96 Plates, Blocks and Tubes, Reagents and Buffers	74181
RNeasy 96 Universal Tissue Kit (4)*	4 RNeasy 96 Plates, Blocks and Tubes, QIAzol Lysis Reagent, Reagents and Buffers	74881
Automated purification of RNA from 1–6 samples		
BioRobot EZ1 [†]	Robotic Workstation, Installation, 1 Year Warranty on Parts and Labor	9000705
EZ1 RNA Cell Mini Kit (48)	Reagent Cartridges, Tips and Tip Holders, Tubes, Buffer, DNase I	958134
EZ1 RNA Tissue Mini Kit (48)	Reagent Cartridges, Tips and Tip Holders, Tubes, Buffer, DNase I	959134
EZ1 RNA Universal Tissue Kit (48)	Reagent Cartridges, Tips and Tip Holders, Tubes, Buffer, QIAzol Lysis Reagent	956136
EZ1 RNA Card	Pre-programmed Card; for use with EZ1 RNA Cell Mini Kit and EZ1 RNA Tissue Mini Kit	9015590
EZ1 RNA Universal Tissue Card	Pre-programmed Card; for use with EZ1 RNA Universal Tissue Kit	9016384

* Larger kit sizes available; for details, visit www.qiagen.com.

[†] QIAGEN robotic systems are not available in all countries; please inquire.

Product	Contents	Cat. no.
Automated purification of RNA from 6–48 samples		
BioRobot M48*	Robotic Workstation, Installation, 1 Year Warranty on Parts and Labor	9000708
MagAttract RNA Cell Mini M48 Kit (192)	MagAttract Suspension E, Buffers, DNase I	958336
MagAttract RNA Tissue Mini M48 Kit (192)	MagAttract Suspension E, Buffers, DNase I	959336
MagAttract RNA Universal Tissue M48 Kit (192)	MagAttract Suspension E, Buffers, QIAzol Lysis Reagent	956336
MagAttract Direct mRNA M48 Kit (192)	MagAttract Suspension C, Buffers	957336
Automated purification of RNA from up to 96 or 192 samples		
BioRobot Gene Expression, Real-Time RT-PCR*	Robotic Workstation and Real-Time RT-PCR Specialist Pack (includes Computer and Software, Starter Kit, Installation and Training, and 1 Year Warranty on Parts and Labor)	9000710
RNeasy 96 BioRobot 8000 Kit (12)	12 RNeasy 96 Plates, Blocks and Tubes, Reagents and Buffers	967152
RNeasy 96 Universal Tissue 8000 Kit (12)	12 RNeasy 96 Plates, Blocks and Tubes, QIAzol Lysis Reagent, Reagents and Buffers	967852
DNase digestion during RNA purification		
RNase-Free DNase Set (50)	RNase-Free DNase I, Buffer RDD, and Water; for 50 minipreps, 25 midipreps, or 17 maxipreps	79254

* QIAGEN robotic systems are not available in all countries; please inquire.

Join the QIAGEN Gene Expression Club

Discover the latest in gene expression research!

The QIAGEN Gene Expression Club reflects a full range of topics, from sample collection to real-time RT-PCR and gene silencing. Look for exciting new developments in the quarterly newsletter!

Sign up now at www.qiagen.com/geneXclub !

Trademarks: QIAGEN®, QIAamp®, BioRobot®, LabelStar™, MagAttract®, MinElute®, Oligotex®, Omniscript®, QuantiTect®, RNAiFect™, RNAprotect™, RNeasy®, Sensiscript® (QIAGEN Group); ABI™, ABI PRISM™ (Applied Biosystems or its subsidiaries); Alexa Fluor™ (Molecular Probes, Inc.); Cy® (Amersham Biosciences); FACS® (Becton, Dickinson and Company); GeneChip® (Affymetrix, Inc.); LightCycler® (Roche Group); PAXgene™, PreAnalytix™ (PreAnalytix GmbH); SAGE™ (Genzyme Corporation); SensiChip™ (Zeplosens).

QIAGEN robotic systems are not available in all countries; please inquire.

Oligotex products are not available in Japan

The SAGE process is covered by U.S. Patent 5,695,937 owned by Genzyme Molecular Oncology.

Purchase of QIAGEN products for PCR containing Taq DNA Polymerase, HotStarTaq DNA Polymerase, or ProofStart DNA Polymerase is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process for research and development activities in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler. The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

The 5' nuclease process is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd.

BioRobot workstations and QIAGEN kits are intended as general-purpose devices. No claim or representation is intended for their use in identifying any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of BioRobot workstations and QIAGEN kits for any particular use, since their performance characteristics have not been validated for any specific organism. BioRobot workstations and QIAGEN kits may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

The PAXgene Blood RNA System is for research use only and not for use in diagnostic procedures.

“RNAlater™” is a trademark of AMBION, Inc., Austin, Texas and is covered by various U.S. and foreign patents.

QIAzol Lysis Reagent is a subject of US Patent No. 5,346,994 and foreign equivalents.

QuantiTect Gene Expression Assays and QuantiTect Custom Assays or portions hereof are subject to proprietary rights of Epoch Biosciences, Inc. and are made and sold under license from Epoch under the patents and patent applications as may be designated by Epoch from time to time set forth, including one or more of the following: U.S. Patent Nos. 5,801,155, 6,084,102, 6,312,894, 6,426,408, and 6,127,121, and applications currently pending. Purchase of this product carries with it a limited, non-transferable, non-exclusive (without the right to resell, repackage, or sublicense) license under U.S. Patent Nos. 6,030,787; 5,723,591; and 5,876,930, and corresponding foreign patents. Powered by innovation from Epoch. Manufactured for QIAGEN by Epoch Biosciences.

© 2004 QIAGEN, all rights reserved.

Gene Expression Analysis

Australia = QIAGEN Pty Ltd

Orders 03-9840-9800 = Fax 03-9840-9888 = Technical 1-800-243-066

Belgium = QIAGEN Benelux B.V.

Orders 0800-79612 = Fax 0800-79611 = Technical 0800-79556

Canada = QIAGEN Inc.

Orders 800-572-9613 = Fax 800-713-5951 = Technical 800-DNA-PREP (800-362-7737)

France = QIAGEN S.A.

Orders 01-60-920-920 = Fax 01-60-920-925 = Technical 01-60-920-930

Germany = QIAGEN GmbH

Orders 02103-29-12000 = Fax 02103-29-22000 = Technical 02103-29-12400

Italy = QIAGEN S.p.A.

Orders 02-33430411 = Fax 02-33430426 = Technical 02-33430414

Japan = QIAGEN K.K.

Telephone 03-5547-0811 = Fax 03-5547-0818 = Technical 03-5547-0811

Luxembourg = QIAGEN Benelux B.V.

Orders 8002-2076 = Fax 8002-2073 = Technical 8002-2067

The Netherlands = QIAGEN Benelux B.V.

Orders 0800-0229592 = Fax 0800-0229593 = Technical 0800-0229602

Switzerland = QIAGEN AG

Orders 055-254-22-11 = Fax 055-254-22-13 = Technical 055-254-22-12

UK and Ireland = QIAGEN Ltd.

Orders 01293-422-911 = Fax 01293-422-922 = Technical 01293-422-999

USA = QIAGEN Inc.

Orders 800-426-8157 = Fax 800-718-2056 = Technical 800-DNA-PREP (800-362-7737)

