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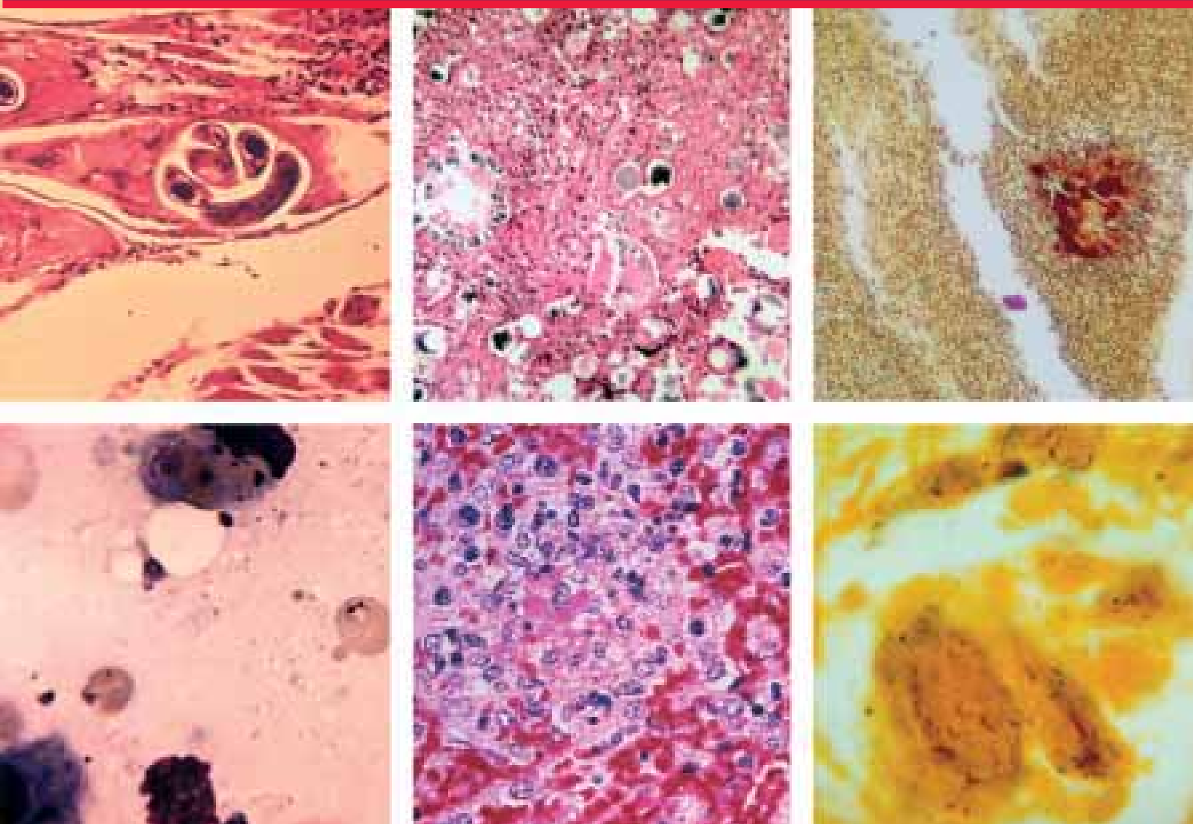
July 2004



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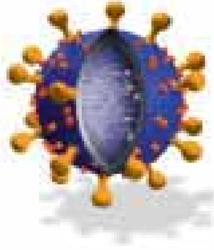
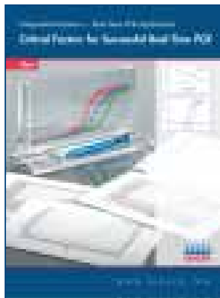


- The new MagAttract® Virus Mini M48 Kit provides a fully automated procedure for simultaneous purification of viral DNA and RNA from serum and plasma with high sensitivity. The kit can be used to purify nucleic acids from a broad range of DNA and RNA viruses. Visit www.qiagen.com/goto/virusM48 to discover how automated viral nucleic acid purification can improve your research!

- HiPerformance Human Library siRNA Sets target complete gene families and contain highly potent, high-purity siRNA duplexes that come with the Golden Guarantee of 100% satisfaction. Custom sets are also available. Find out more at www.qiagen.com/siRNA .

- Visit www.qiagen.com/siRNA for the latest information on a regularly updated range of validated siRNA sequences. The sequences are selected from peer-reviewed scientific publications, and are supplied with full documentation, including GenBank® references, literature citations, and mRNA target sequences.

- Find the QIAGEN plasmid kit to suit your exact needs using the new online selection guide at www.qiagen.com/goto/plasmidselection ! QIAGEN provides the most comprehensive range of plasmid purification kits available — from mini- to gigapreps or service production, and from convenient manual formats to walkaway automated systems. Now it is even easier to find the best kit for you!



- *Critical Factors for Successful Real-Time PCR* is an indispensable guide that provides information and hints and tips on all aspects of the real-time PCR process. Visit www.qiagen.com and download your free copy.

- Coming soon! The QuantiTect® Multiplex PCR Kit enables real-time amplification and detection of multiple target sequences without the need for optimization.

- Visit www.qiagen.com/goto/assays for up-to-date information on QuantiTect® Gene Expression Assays or to design your own. The list of available assays is regularly updated as new ones become available.

- QIAGEN is pleased to announce a new subsidiary providing direct service and support in Benelux. Visit www.qiagen.com or see page 55 for contact details.

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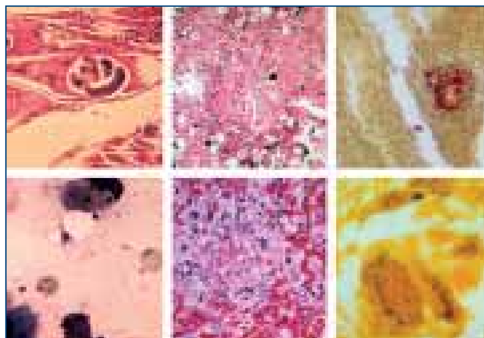


Cover New RNeasy® 96 Universal Tissue Kits provide high-throughput RNA purification from all types of animal or human tissue, including (from upper left to lower right): muscle, brain, testicular tissue, liver, spleen, and kidney. See page 33 for more information. (Tissue micrographs were obtained from the Centers for Disease Control and Prevention [CDC], and were produced by the CDC, Neva Gleason, Dr. Martin Hicklin, and Dr. Marshall Fox. Use of these images does not imply endorsement by the CDC or any CDC employee.)

More precise results from Affymetrix® GeneChip® arrays

The BioRobot® Gene Expression system with the GeneChip Target Preparation Specialist Pack is a complete automated solution for preparing labeled cRNA targets for use with Affymetrix GeneChip arrays. By automating the steps from first-strand cDNA synthesis to fragmentation of cRNA, the system both saves hands-on time and standardizes the preparation of cRNA targets, enabling more precise GeneChip array results.

The BioRobot Gene Expression — GeneChip Target Prep system provides:

- **More precise GeneChip array analyses** — standardized target preparation, from cDNA synthesis to cRNA fragmentation
- **Reduced hands-on time** — automated target-preparation process
- **Comprehensive support** — including installation, training, chemistries starter pack, and software protocols

Complete automation and support

The system comprises a robotic workstation that is integrated via a robotic arm with external hardware required for the GeneChip target-preparation process. The entire system is operated by a computer installed with QIAsoft 4.2 Operating System and CLARA™ scheduling software.

Immediate startup of the system and continued success is assured through the specialist pack, which includes installation and training, external hardware, operating software, accessories, and chemistries starter pack. Certified software protocols supplied with the specialist pack allow easy system operation and reproducible processing of samples.

High-quality cRNA targets

The system processes up to 96 samples, in multiples of 8, per run. Starting with 5 µg total RNA per sample, the system generates cRNA targets with yields of at least 20 µg and concentrations of at least 0.625 µg/µl. cRNA targets are pure, as determined by absorbance measurements, with A_{260}/A_{280} ratios greater than 1.8, and intact, as determined by analysis using the Agilent 2100 bioanalyzer (Figure 1).

High-quality array data

Standardized sample processing enables high-quality array data, as determined by measurements of percentage present call, background, and 3'/5' ratio for GADPH and actin (Figure 2). Additionally, array data obtained from cRNA targets prepared using the system correlate well with array data obtained from cRNA targets prepared using a manual procedure (Table 1). ►

High-Quality cRNA Targets

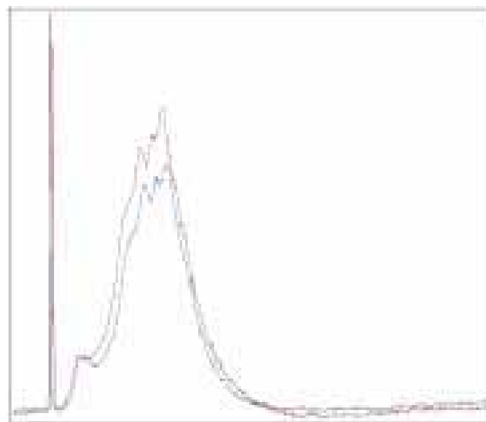


Figure 1 Total RNA was purified from HeLa S3 cells using the RNeasy® Midi Kit. Ninety-six RNA samples (5 µg each) were processed on the BioRobot Gene Expression workstation. The cRNA targets generated were analyzed using the Agilent 2100 bioanalyzer. A representative sample (brown) was compared with a sample prepared using a manual procedure (blue).

Table 1. High Correlation with Manual Procedure

Well	Pearson correlation coefficient (*r*)	r^2
E7	0.991	0.983
E9	0.995	0.990
F1	0.996	0.992
F4	0.988	0.976
G12	0.997	0.994
H6	0.994	0.988
H8	0.997	0.994

cRNA samples were prepared and hybridized as described in Figure 2. The signal values for 7 cRNA samples were correlated with the signal values from a cRNA sample prepared using a manual procedure.

High-Quality Array Data

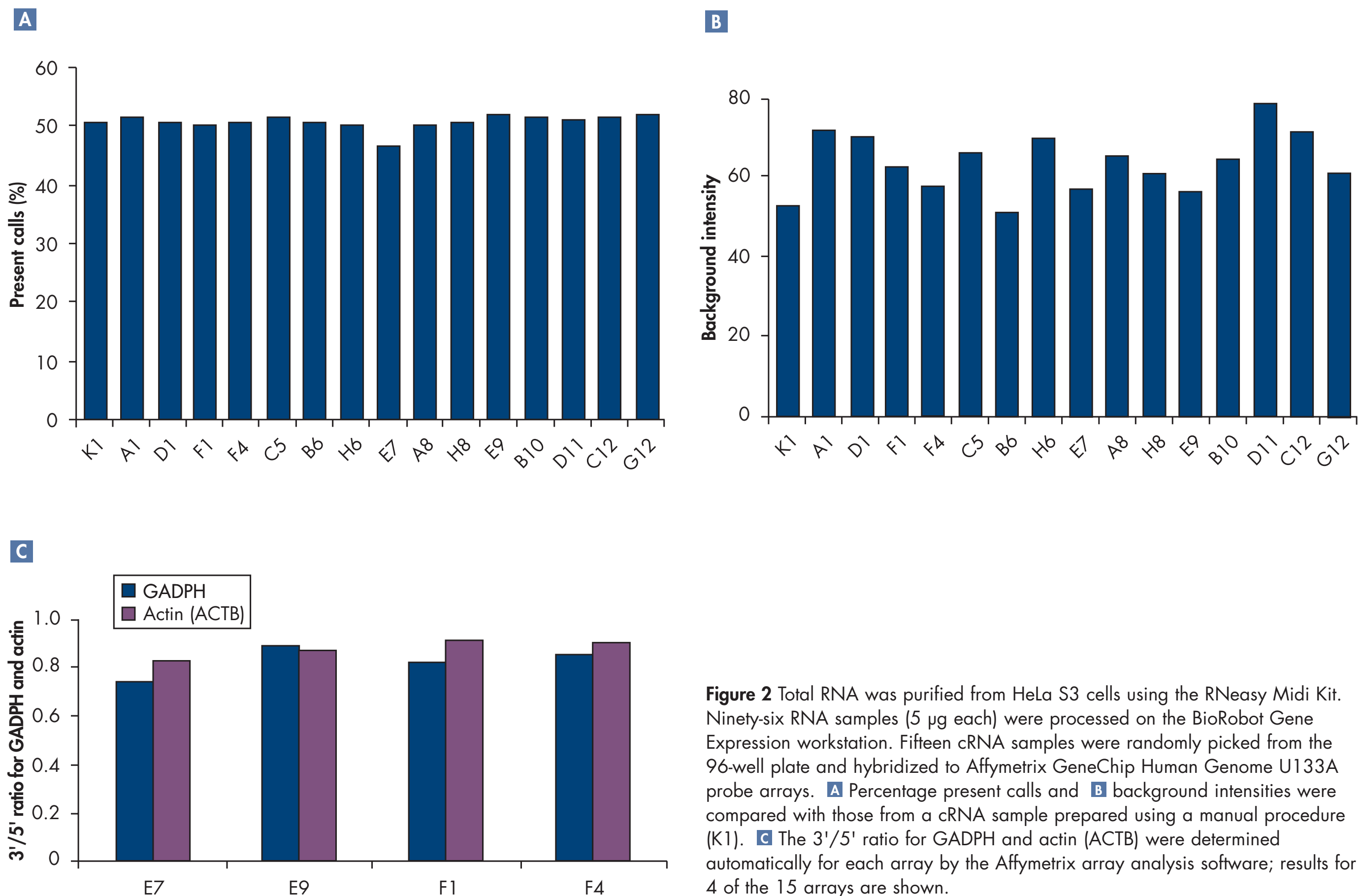


Figure 2 Total RNA was purified from HeLa S3 cells using the RNeasy Midi Kit. Ninety-six RNA samples (5 µg each) were processed on the BioRobot Gene Expression workstation. Fifteen cRNA samples were randomly picked from the 96-well plate and hybridized to Affymetrix GeneChip Human Genome U133A probe arrays. A Percentage present calls and B background intensities were compared with those from a cRNA sample prepared using a manual procedure (K1). C The 3'/5' ratio for GADPH and actin (ACTB) were determined automatically for each array by the Affymetrix array analysis software; results for 4 of the 15 arrays are shown.

Conclusion

The BioRobot Gene Expression system standardizes the preparation of high-quality cRNA for more precise results from Affymetrix GeneChip arrays.

Ordering Information

Product	Contents	Cat. no.
BioRobot Gene Expression, GeneChip Target Prep*	Robotic workstation and GeneChip Target Preparation Specialist Pack, including BioRobot Twister® II, spectrophotometer, thermocycler, QIAsoft 4.2 Operating System, CLARA scheduling software, chemistries starter kit, QIAsoft protocols, installation, training, and 1 year warranty on parts and labor	9000821

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

High-throughput RNA purification from all types of animal tissue

The RNeasy® 96 Universal Tissue Kit provides parallel purification of total RNA from up to 96 or 192 samples of all types of animal or human tissue, including difficult-to-lyse fibrous and fatty tissues.

Benefits of the RNeasy 96 Universal Tissue Kit:

- **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 manual or automated protocol
- **Pure, high-performance RNA** — without phenol contamination
- **High-quality RNA for all downstream applications** — such as real-time RT-PCR and array analysis

High yields of total RNA from all types of tissue in 96-well format

The RNeasy 96 Universal Tissue procedure enables high-throughput purification of RNA from any tissue sample, including difficult-to-lyse fibrous and fatty tissues (Figure 1). Compared with standard silica-membrane procedures, the RNeasy 96 Universal Tissue Kit provides higher yields of total RNA for all tissue types (Table 1).

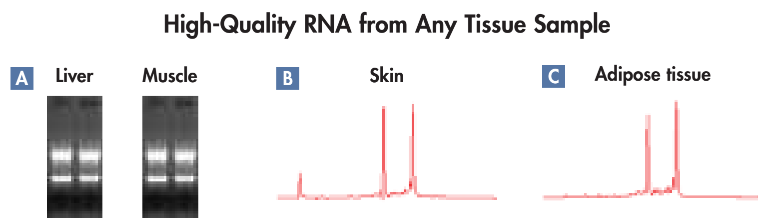
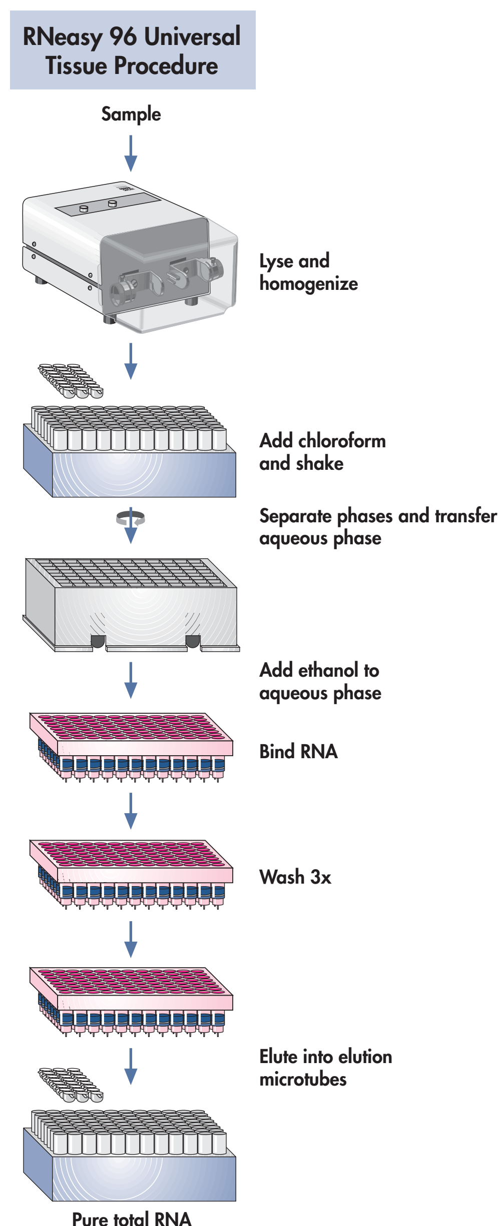


Figure 1 Total RNA was purified from the indicated rat tissues using the RNeasy 96 Universal Tissue Kit and analyzed by formaldehyde agarose gel electrophoresis or on the Agilent 2100 bioanalyzer. Starting materials were A 25 mg RNA*later*™ stabilized liver and muscle, B 50 mg flash-frozen skin, and C 100 mg flash-frozen adipose tissue.

Automated protocol on the BioRobot® Gene Expression workstation

An automated format of the RNeasy 96 Universal Tissue Kit is available for use with the BioRobot Gene Expression — Real-Time RT-PCR system. All steps from transfer of the aqueous phase to elution of pure RNA can be performed by the workstation. ▶



Integrated protocol with TissueLyser disruption, QIAzol lysis, and RNeasy purification

Efficient high-throughput disruption using the TissueLyser system provides parallel disruption and homogenization, with lysis of up to 2 x 96 samples in QIAzol Lysis Reagent. The combination of QIAzol and RNeasy technologies results in highly pure RNA without phenol carryover. RNA purified using the RNeasy 96 Universal Tissue Kit is suitable for all downstream applications, including real-time RT-PCR (Figure 2) and array analysis.

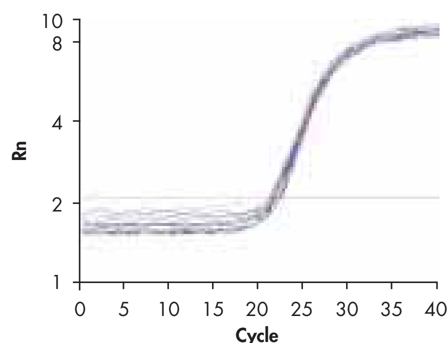


Figure 2 Total RNA was purified from 50 mg samples of rat brain using the RNeasy 96 Universal Tissue Kit. Twenty-four purified samples, with an average of 90 ng RNA per reaction, were analyzed by real-time RT-PCR on the ABI PRISM® 7900HT Sequence Detection System, using the QuantiTect® Probe RT-PCR Kit with primers and probe specific for the c-jun gene.

Table 1. Typical Total RNA Yields Using the RNeasy 96 Universal Tissue Kit

Tissue	RNA yield (µg per 10 mg of tissue)
Liver	15–80
Heart	5–25
Skin	2–5
Brain	5–20
Adipose tissue	0.5–2.5

Related article in this issue

Easy-to-use automation for RNA purification (page 35)

Ordering Information

Product	Contents	Cat. no.
RNeasy 96 Universal Tissue Kit (4)*	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Plasticware, RNase-Free Reagents and Buffers	74881
RNeasy 96 Universal Tissue Kit (12)*	For 12 x 96 total RNA preps: 12 RNeasy 96 Plates, Plasticware, RNase-Free Reagents and Buffers	74882
RNeasy 96 Universal Tissue 8000 Kit (12)*	For 12 x 96 total RNA preps on the BioRobot Gene Expression — Real-Time RT-PCR system: 12 RNeasy 96 Plates, Plasticware, RNase-Free Reagents and Buffers	Inquire

* Requires use of the Plate Rotor 2 x 96 and Centrifuge 4K15C (TissueLyser system recommended for disruption and homogenization; QIAvac 96 optional).

Easy-to-use automation for RNA purification

BioRobot® EZ1 and M48 workstations deliver ease of use and increased reproducibility for life-science and clinical researchers purifying total RNA and mRNA from a wide range of cell and tissue samples. The BioRobot EZ1 provides rapid, automated processing of 1–6 samples per run while the BioRobot M48 offers more flexibility and walkaway processing of 6–48 samples per run.

BioRobot EZ1 and M48 systems provide:

- **Easy and reproducible purification of RNA** — from 1–48 cultured cell or tissue samples per run using fully automated magnetic-particle technology
- **RNA that enables sensitive gene expression analysis** — including microarray analysis or real-time RT-PCR
- **Linear yields of total RNA** — from a range of cell numbers
- **Choice** — choose easy-to-use EZ1 protocol cards with pre-filled and sealed reagent cartridges or flexible MagAttract® M48 Kits

Easy, hands-free RNA purification

BioRobot EZ1 and M48 workstations use silica-coated MagAttract magnetic particles to purify RNA. MagAttract technology eliminates tedious centrifugation steps and provides high-performance RNA suitable for sensitive downstream applications. Pre-sealed reagent cartridges and the workstation door combine to reduce the chances of environmental contamination or interference due to RNases when using the BioRobot EZ1 workstation. Adjustable elution volumes allow RNA yields from cells or tissues to be optimized for a range of applications when using the BioRobot M48 workstation. The BioRobot M48 workstation provides protocols for total RNA purification and for direct purification of mRNA. ►

Table 1. Efficient RNA Purification and Consistent Real-Time RT-PCR Analysis from Human Breast Carcinomas

Sample	A_{260}/A_{280} ratio	RNA concentration (ng/µl)	Mean C_T
Ductal carcinoma samples from human breast tissue			
1	1.9	36.7	24.0 (± 0.2)
2	1.9	64.5	22.8 (± 0.1)
3	1.9	32.1	24.4 (± 0.2)
4	2.0	39.8	25.4 (± 0.2)
5	2.0	23.2	23.9 (± 0.1)
Sarcoma sample from human breast tissue			
6	1.8	30.7	23.9 (± 0.02)

Ductal carcinoma samples were homogenized immediately after sampling and stored at –70°C for 8 weeks before processing on the BioRobot M48 workstation. The sarcoma sample was homogenized and processed on the BioRobot M48 workstation immediately after sampling. Real-time RT-PCR (25 µl) of human β-glucuronidase mRNA was performed in triplicate using 5 µl purified RNA per reaction. (Data kindly provided by J. Delabie and B. Risberg, Norwegian Cancer Hospital, Oslo, Norway.)

High-quality RNA ensures precise analysis

The high purity and integrity of RNA purified using BioRobot EZ1 and M48 systems (Figure 1) ensures high performance in gene expression analyses. The high-quality RNA allows sensitive and precise downstream analysis using methods such as microarray analysis (Figure 2) and real-time RT-PCR (Table 1). Up to 1 x 10^6 cultured cells, 2 x 10^6 blood cells, or 10 mg easy-to-lyse tissue can be processed per sample. Yields range from 15 to 40 µg RNA per sample, depending on sample type and amount.

Reproducible Purification of High-Quality RNA

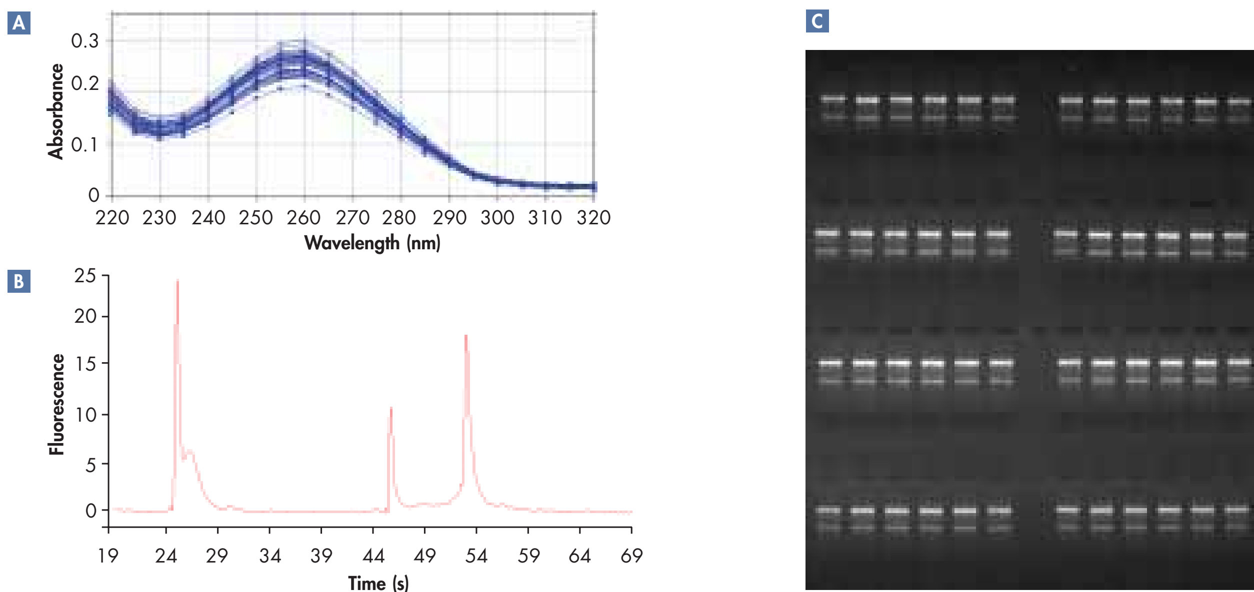


Figure 1 RNA was purified from 48 samples of 1 x 10^6 HeLa cells each using the BioRobot M48 workstation and the MagAttract RNA Cell Mini M48 Kit. Purified RNA was eluted in a volume of 200 µl. A Absorbance of purified RNA shows consistent high purity. B Agilent 2100 bioanalyzer analysis of 1 µl purified RNA from a representative sample. C Aliquots (20 µl) of purified RNA, visualized on an agarose gel, show intact ribosomal RNA bands.

Efficient Labeling of Target for Microrray Analysis

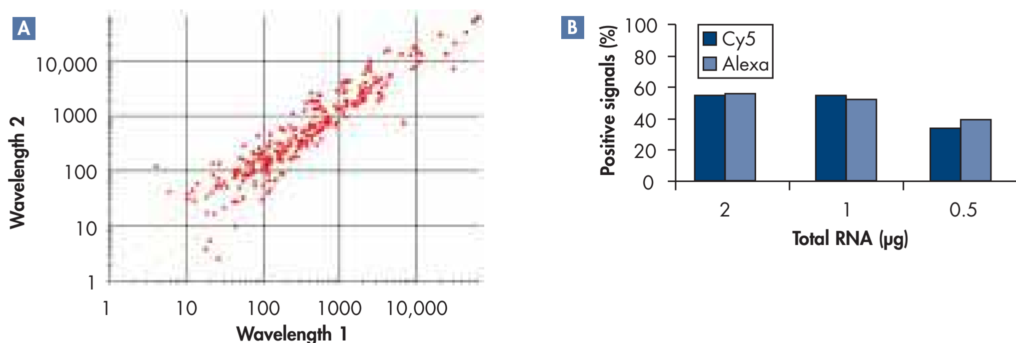


Figure 2 Total RNA was purified from HeLa cells using the BioRobot EZ1 system. cDNA was synthesized from total RNA and simultaneously labeled with both Alexa Fluor® 532 and Cy®5 fluorophores using the QIAGEN LabelStar™ Array Kit. Labeled cDNA was hybridized to a SensiChip™ DNA Array Bar containing stress- and aging-specific capture probes. A Plot showing that intensities of signal correlate well following hybridization with 1 µg cDNA, independent of the fluorophore type. B Comparison of numbers of positive (signal:noise ratio >3) signals detected (5-second exposure) following hybridization with the indicated amounts of total RNA.

Reproducible RNA yields ensure more meaningful data

BioRobot M48 and EZ1 kits provide efficient and standardized purification of RNA. The reproducible efficiency of RNA purification using EZ1 and MagAttract kits is reflected by the low variation observed in real time RT-PCR of both low- and high-abundance mRNAs (Figure 3). Reproducible purification (Figure 4), using these kits, limits variability in yields and provides a solid foundation for sensitive techniques such as real-time RT-PCR (Figure 5), where even small variations in extraction efficiency or RNA quality can bias results.

Repeatable Accuracy with High- to Low-Copy mRNAs

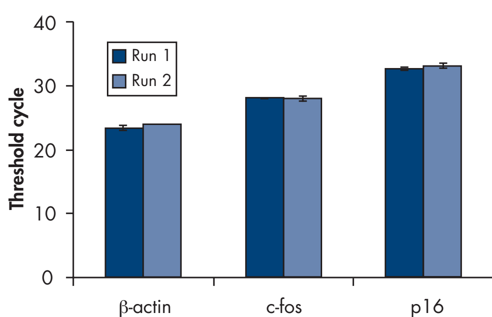


Figure 3 Total RNA was purified from 1 x 10^6 HeLa cells. Purified RNA was eluted in 200 µl, and 5 µl aliquots were used in 25 µl real-time RT-PCR of human β-actin, c-fos, and p16 mRNA using the QuantiTect® Probe RT-PCR Kit. Three reactions were performed using purified RNA from each of 2 consecutive runs.

Reproducible RNA Yields from a Range of Tissues

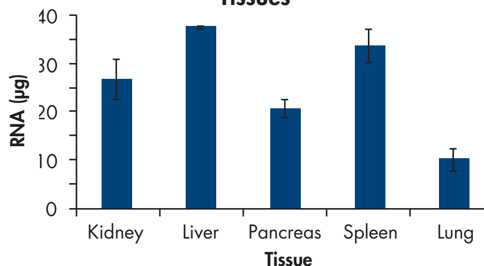
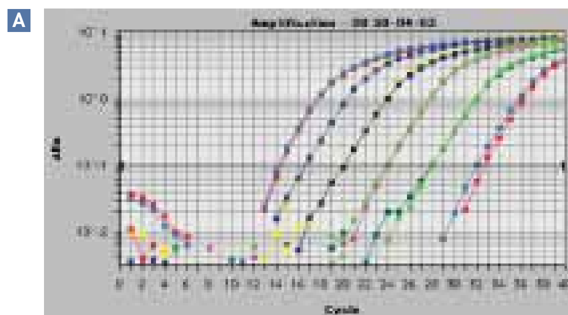


Figure 4 Total RNA was purified from 10 mg samples of rat kidney and liver and from 5 mg samples of rat pancreas, spleen, and lung. RNA was purified from 3 replicate samples of each tissue type. Yields of total RNA were determined by absorbance (A_{260}) with correction for background (A_{320}). CV Kidney = 15%, CV Liver = 1%, CV Pancreas = 8%, CV Spleen = 10%, CV Lung = 22%.

Linear C_T Values over a Large Dynamic Range

A



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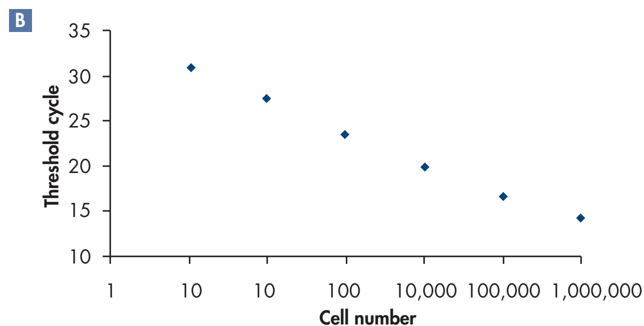


Figure 5 Tenfold dilution series were performed on lysates of 1 x 10^6 HeLa cells to produce aliquots equivalent to 1 x 10^1–10^6 cells. RNA was purified from lysates using the BioRobot EZ1 system. Purified RNA was eluted in a volume of 200 µl and aliquots of 5 µl were used in 25 µl real-time RT-PCR of human c-myc mRNA using the QuantiTect Probe RT-PCR Kit. **A** ABI® 7700 real-time amplification plots. **B** Plot of C_T values and cell number equivalents.

Related article in this issue

High-throughput RNA purification from all types of animal tissue (page 33)

Visit www.qiagen.com today to discover more about easy solutions for reproducible purification of high-performance RNA!

For ordering information, see page 48.

New

BioSprint systems — fast and economical sample preparation

The new range of BioSprint workstations and kits provide fast and cost-efficient sample preparation using magnetic-particle technology. BioSprint technology is highly adaptable so that BioSprint workstations have the potential to purify different types of target molecules from different types of samples. The first kits to be introduced are for genomic DNA purification from blood and blood products. Kits for other sample-preparation procedures are coming soon.

The BioSprint 15 Workstation

The BioSprint 96 Workstation

BioSprint workstations and kits provide:

- **Adaptability to specific needs** — the open design of the workstations allows compatibility with QIAGEN® procedures or protocols developed by the user*
- **Variable throughput** — processing 1–15 or 1–96 samples per run, and hundreds of samples per day
- **A cost-efficient solution** — economically priced automation saves time and effort
- **Rapid sample processing** — a unique automated procedure in which magnetic particles are transferred between solutions enables fast purification

Flexible and customizable automation

Two workstations with different throughputs are available: the BioSprint 15 and the BioSprint 96 for processing 1–15 and 1–96 samples per run, respectively. Both workstations come preinstalled with protocols that enable sample preparation using BioSprint kits. Users also have the option of writing their own protocols using the BioSprint software, allowing adaptation of the workstations to specific or changing research needs.

Easy-to-use, economical automation

Each BioSprint workstation is an open system with a small footprint and is easily operated through a touchpad keyboard. Because of the minimal number of mechanical components, the workstations are both easy to install and economically priced. BioSprint kits provide all the necessary reagents and plasticware for sample preparation and are available in different sizes, enabling cost-efficient use of consumables.

Efficient Processing of Magnetic Particles

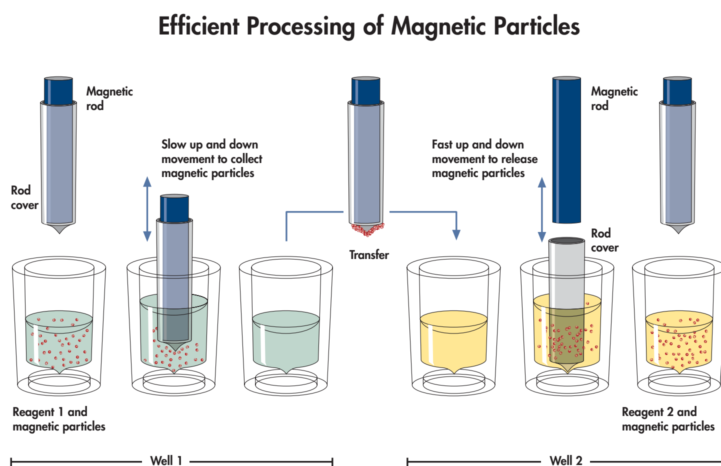


Figure 1 The BioSprint workstation transfers magnetic particles from one well or tube to another.

* To create and add new protocols, a PC (used with BioSprint software) is required.

Rapid sample preparation

By automating the simultaneous preparation of up to 15 or 96 samples, BioSprint workstations save both time and effort. Each workstation controls an array of magnetic rods that can attract or release magnetic particles and transfer them from tube to tube or from well to well. The sequential transfer of magnetic particles allows a rapid purification procedure to be performed, from the initial binding of target molecules (e.g., genomic DNA) to the particles, through to washing of the particles and elution of pure target molecules. Since the workstations transfer magnetic particles instead of liquids, they use minimal amounts of reagents, enabling cost-efficient sample preparation (Figure 1).

Support for a wide range of applications

QIAGEN will introduce a range of BioSprint Kits for use with different sample types and for purification of different types of target molecule. The first kits available are the BioSprint DNA Blood Kits, which enable purification of genomic DNA from blood or blood-related products. Lysed samples are loaded into the workstation, which then performs all steps until pure genomic DNA is obtained. The high-quality DNA performs well in downstream applications, including PCR (Figure 2).

Conclusion

The BioSprint workstations and the expanding range of BioSprint Kits provide research labs with fast, economical, and flexible sample preparation.

Efficient PCR Performance of DNA Purified Using the BioSprint 15 DNA Blood Kit

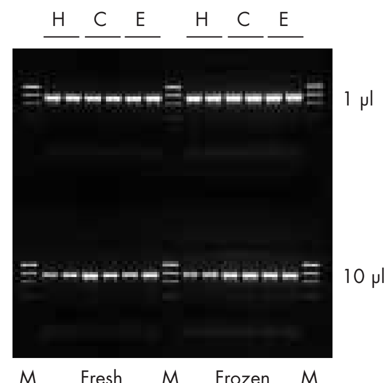


Figure 2 Blood was collected and treated with one of 3 anticoagulants: heparin (**H**), citrate (**C**), or EDTA (**E**). DNA was purified from 200 µl blood immediately after blood collection (**Fresh**) and after one cycle of freezing and thawing (**Frozen**). DNA was eluted in 200 µl elution buffer. Aliquots (1 µl and 10 µl) were added to PCR (50 µl reaction volume) using primers to amplify a 1 kb fragment of the Hugl gene. PCR products were run on a 1.5% agarose gel in 1x TBE. **M**: markers.

Ordering Information

Product	Contents	Cat. no.
BioSprint 15*	Robotic workstation for automation of magnetic-particle purification technology	9000850
BioSprint 96*	Robotic workstation for automation of magnetic-particle purification technology	9000852
BioSprint 15 DNA Blood Kit (360)†	5-Rod Covers, 5-Tube Strips, MagAttract Suspension G, Buffers and Reagents	940017
BioSprint 96 DNA Blood Kit (384)†	Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	940057

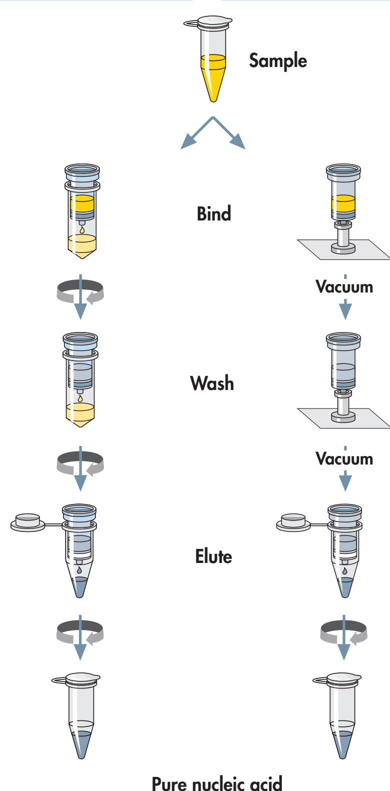
* QIAGEN robotic systems are not available in all countries, please inquire.

† Smaller kit sizes available; please visit www.qiagen.com .

New

Streamline your spin preps with the new QIAvac 24 Plus vacuum manifold!

The new QIAvac 24 Plus vacuum manifold enables fast and efficient vacuum processing of QIAGEN® spin columns in both clinical and laboratory research. Vacuum-driven liquid processing is an attractive alternative to centrifugation that minimizes the hands-on time needed for sample preparation.



The new QIAvac 24 Plus offers:

- **Time savings** — rapid vacuum-driven liquid processing
- **Convenience** — minimal hands-on-time
- **Flexibility** — process up to 24 spin columns in parallel

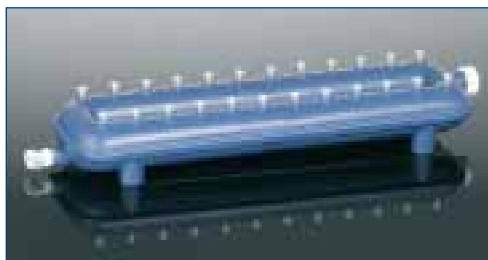
Table 1. QIAGEN Kits Compatible with the QIAvac 24 Plus*

Research kits	Clinical research kits	CE-certified DSP kits
QIAprep® Miniprep Kit	QIAamp® DNA Blood Kits†	QIAamp DSP DNA Blood Mini Kit‡
QIAquick® PCR Purification Kit	QIAamp DNA Mini Kit†	QIAamp DSP Virus Kit‡
QIAquick Gel Extraction Kit	QIAamp Viral RNA Mini Kit†	
QIAquick Nucleotide Removal Kit	QIAamp MinElute Virus Vacuum Kit†	
MinElute™ PCR Purification Kit		
MinElute Gel Extraction Kit		
MinElute Reaction Cleanup Kit		
RNeasy® Mini Kit		

* The QIAvac 24 Plus is intended for general laboratory use. No claim or representation is intended for its use to identify 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 the QIAvac 24 Plus for any particular use, since its performance characteristics have not been validated for any specific organism. The QIAvac 24 Plus 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. Performance evaluation studies with the respective QIAamp DSP CE-certified kits were carried out to qualify the QIAvac system for the kit specific intended use.

† QIAamp Kits are intended as general-purpose devices that 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.

‡ Only available in Austria, Belgium, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, the Netherlands, Portugal, Spain, Switzerland, and the United Kingdom.



The QIAvac 24 Plus vacuum manifold.

Reduced sample handling

Vacuum-driven liquid processing facilitates sample handling and reduces hands-on preparation time by eliminating the tedious loading and unloading of spin columns into a centrifuge and subsequent removal of flow-through during binding and washing procedures (see flowchart). Up to 24 spin columns can be processed simultaneously using the QIAvac 24 Plus vacuum manifold.

Easier and faster sample processing

The QIAvac 24 Plus provides easier and faster handling of spin columns than using centrifugation. Simply insert 1–24 spin columns into the manifold, add the samples or buffers, and turn on the vacuum source. Samples and buffers are drawn efficiently through the spin columns, facilitating multiple loadings of large sample volumes and eliminating the need to discard flow-through. Vacuum-driven liquid processing is faster than centrifugation and still gives the same high, reproducible yields.

Clinical and research applications

In combination with the QIAvac Connecting System (Figure 1), the QIAvac 24 Plus can be used as a flow-through system. The sample flow-through, containing possibly infectious material, is collected in a separate waste bottle.

Prevention of cross-contamination is especially critical when purifying nucleic acids from clinical samples. QIAGEN spin columns are inserted into disposable VacConnectors, preventing direct contact of the spin column with the manifold. VacValves ensure constant vacuum pressure with viscous samples like blood. The QIAvac 24 Plus proved to be an ideal tool in studies evaluating the performance of QIAGEN's new CE-certified clinical diagnostic kits.

Integrated System for Research and Clinical Applications

Figure 1 The QIAvac 24 Plus, Connecting System, and Vacuum Pump allows collection of infectious material for clinical applications.

With the QIAvac 24 Plus vacuum manifold system, processing nucleic acid spin columns has never been so easy!

Ordering Information

Product	Contents	Cat. no.
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, Quick Couplings	19413
Vacuum Pump (230 V, 50 Hz)	Universal vacuum pump (capacity 34 L/min, 8 mbar vacuum abs., 230 V, 50 Hz)	84020
QIAvac Connecting System	System to connect vacuum manifold with vacuum pump: includes Tray, Waste Bottles, Tubings, Couplings, Valve, Gauge, 24 VacValves	19419
VacValves (24)	24 valves for use with the QIAvac 24 Plus	19408
VacConnectors (500)	500 disposable connectors for use with QIAamp spin columns on luer connectors	19407

New

Guaranteed efficient gene silencing using 2-for-Silencing siRNA Duplexes

Designed using the revolutionary HiPerformance algorithm and stringent homology analysis, 2-for-Silencing siRNA Duplexes deliver guaranteed knockdown of target genes, providing optimal results with less effort and expense.

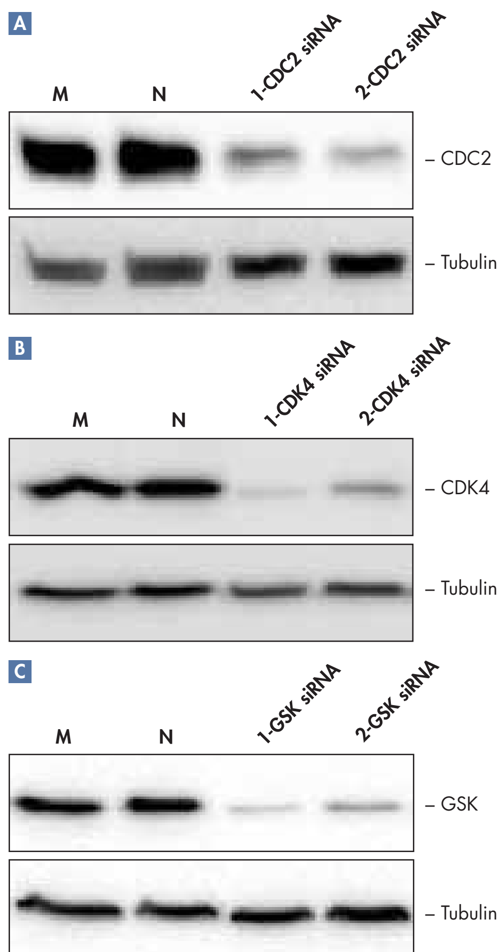


Figure 1 HeLa S3 cells were transfected with siRNA targeted against each of three genes: A CDC2, B CDK4, and C GSK using RNAiFect™ Transfection Reagent. Cells were also mock transfected without siRNA addition (**M**) and transfected with a non-silencing control siRNA (**N**). After 48–72 hours, cell lysates were separated by SDS-PAGE and analyzed on a western blot using CDC2-, CDK4-, or GSK-specific antibodies. Blots were also probed with tubulin-specific antibody, as an internal control.

With 2-for-Silencing siRNA Duplexes you get:

- **Guaranteed silencing to your satisfaction** — highly efficient knock-down with at least one of the two duplexes is assured
- **Revolutionary design** — using the HiPerformance design algorithm licensed from Novartis Pharmaceuticals
- **Fast and economical silencing** — screening just two siRNAs saves time and expense

Highly efficient gene silencing

2-for-Silencing siRNAs guarantee effective knockdown with at least one of the two duplexes supplied. Western blot analysis shows the high level of knockdown achieved using 2-for-Silencing duplexes directed against three genes (Figure 1). The results show highly efficient silencing using siRNAs designed using the HiPerformance algorithm.

siRNA design using the revolutionary HiPerformance algorithm

Rational siRNA design and stringent homology analysis are critical for achieving optimal silencing of target genes, and for minimizing off-target effects. QIAGEN has licensed the HiPerformance design algorithm from Novartis Pharmaceuticals for the selection of highly functional target sequences for RNAi. The algorithm is based on the largest independent study of siRNA functionality to date, in which the gene silencing efficiency of more than 3000 synthetic siRNA duplexes, directed against 34 targets, was analyzed.

The HiPerformance design algorithm is integrated with a proprietary homology analysis tool that has sensitivity equal to that of the Smith-Waterman algorithm, but is much faster. A comprehensive, in-house, non-redundant gene database has been developed that allows thorough and accurate homology analysis (see flowchart).

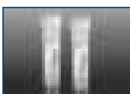
Design of 2-for-Silencing siRNA Duplexes

Entire mRNA surveyed
All possible 21mers of a target gene sequence are analyzed for potential RNAi activity using the HiPerformance design algorithm

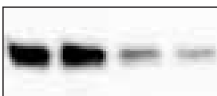
Stringent homology analysis
All high-scoring candidates are ranked by uniqueness using a sensitive alignment tool and a non-redundant sequence database

siRNA sequence selection
Two siRNAs that combine optimal potential activity and high specificity are chosen

Product
2-for-Silencing siRNA Duplexes



Performance
Guaranteed gene knockdown



High-purity siRNA synthesis

2-for-Silencing siRNA Duplexes are delivered as 20 nmol of HPP (High-Performance Purity) Grade siRNA. HPP Grade siRNA is >90% pure, eliminating the time and expense needed for HPLC or PAGE purification. The novel synthesis process improves yields and high coupling efficiency ensures that siRNA is full length. The two duplexes are supplied separately, and complete sequence information is provided at no extra cost. There are no restrictions on publication of sequence information.

Related article in this issue

Efficient DNA and siRNA transfection of primary cells (page 44)

Make a breakthrough in your RNAi research with 2-for-Silencing siRNA Duplexes — find out more at www.qiagen.com/siRNA !

Ordering Information

Product	Contents	Cat. no.
2-for-Silencing siRNA Duplexes	Two HPP Grade siRNAs (20 nmol), custom-designed by QIAGEN	1022562

Efficient DNA and siRNA transfection of primary cells

Jörg Dennig, Juliane Konrad, Silvia Magyar, Ute Krüger, Wolfgang Bielke, and Jie Kang

QIAGEN GmbH, Hilden, Germany

Primary cells are often preferred to cultured cell lines for investigations where it is important for the cells to be as similar as possible to cells in vivo. Therefore, their role in cell biology is becoming increasingly important. Unfortunately, preparation of primary cells and their cultivation can be a challenge, and transfection with either DNA or siRNA can also be difficult in some cell types. Effectene® Transfection Reagent and RNAiFect™ Transfection Reagent — chosen for their low cytotoxicity and high efficiency of transfection in a broad range of cell lines — were tested for transfection of commonly used primary cell types.

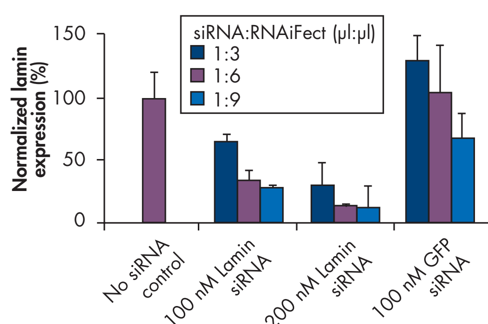


Figure 1 Gene silencing of lamin A/C in HMEC.

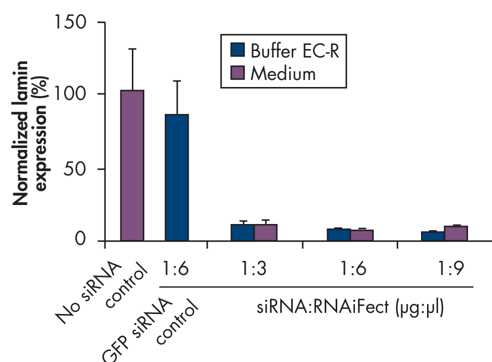


Figure 2 Gene silencing of lamin A/C in HAEC. siRNA (100 nM) was added to cells using the indicated ratio of siRNA to RNAiFect Reagent and either Buffer EC-R or complete medium for complex formation.

Materials and methods

Human mammary epithelial cells (HMEC) were transfected with two different concentrations of an siRNA targeted against lamin A/C using RNAiFect Transfection Reagent. Cells were transfected in 24-well plates following the protocol described in the *RNAiFect Transfection Handbook*. Buffer EC-R was used for complex formation, and the complexes were removed after overnight incubation. After 2 days, lamin A/C expression was determined after purification of RNA using the RNeasy® Mini Kit and RT-PCR using the QuantiTect® Probe RT-PCR Kit. Lamin A/C expression was normalized to expression of the housekeeping gene, GAPDH. An siRNA targeted against GFP (green fluorescent protein) was used as a non-silencing control. In a second series of experiments, human aortic endothelial cells (HAEC) were transfected with siRNA. Complex formation in medium was compared with complex formation in Buffer EC-R for siRNA transfection using 100 nM siRNA.

To test the efficiency of DNA transfection into primary cells, human umbilical vein endothelial cells (HUVEC) and HAEC were transfected with a green fluorescent protein (GFP) expression plasmid using Effectene Transfection Reagent according to the protocol described in the *Effectene Transfection Reagent Handbook*. Cells were transfected in 6-well plates using 0.4 µg DNA with Enhancer at a ratio of 1:8. Complexes were removed after 6 hours, and cells were analyzed by FACS® two days post-transfection.

Gene silencing in primary epithelial and endothelial cells

In a series of optimization experiments in primary epithelial cells, the highest level of silencing of lamin A/C expression was obtained using 200 nM siRNA and 1:6 or 1:9 ratios of RNAiFect Reagent (Figure 1). In primary endothelial cells, lamin expression knockdown of approximately 90% was obtained using all tested ratios of siRNA to transfection reagent (Figure 2). Gene silencing was found to be equally effective, in the cell type tested, when using medium or Buffer EC-R.

DNA transfection of human primary endothelial cells

To determine DNA transfection efficiency in two human primary endothelial cell types, human primary umbilical vein and aortic endothelial cells were transfected with a GFP expression plasmid using Effectene Transfection Reagent. Transfection efficiencies were between 20 and 30% (Figure 3).

Transfection of primary neurons

TransMessenger® Transfection Reagent, originally developed for RNA transfection of eukaryotic cells, has previously been shown to be effective for DNA and siRNA transfection of primary neurons (1, 2).

Summary

- Gene silencing was effective in two different primary cell types using siRNA and RNAiFect Transfection Reagent from QIAGEN.
- DNA transfection was efficient in two different primary cell types using a GFP expression plasmid and Effectene Transfection Reagent.
- QIAGEN transfection reagents provide a choice of solutions for transfection of primary cell types.

Related article in this issue

Guaranteed efficient gene silencing using 2-for-Silencing siRNA Duplexes (page 42)

Efficient DNA Transfection of Human Endothelial Cells

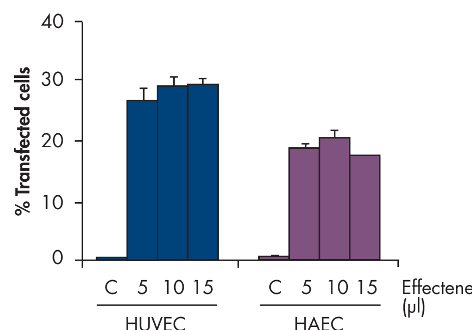


Figure 3 DNA transfection in human endothelial cells. Mean values are given for duplicate wells. HUVEC and HAEC were transfected in 6-well plates using 0.4 µg GFP DNA and the indicated amount of Effectene Transfection Reagent. **C**: untransfected cells.

References

1. Narz, F., Janhsen, S., and Krüger, U. (2003) Efficient DNA transfection of primary CNS neurons using TransMessenger Transfection Reagent. QIAGEN News **2003**, e6.
2. Krichevsky, A.M. and Kosik, K.S. (2003) RNAi functions in cultured mammalian neurons. Proc. Natl. Acad. Sci. USA **99**, 11926.

Ordering Information

Product	Contents	Cat. no.
Effectene Transfection Reagent (1 ml)*	1 ml Effectene Reagent, Enhancer, Buffer; for 40 transfections in 60 mm dishes or 160 transfections in 12-well plates	301425
RNAiFect Transfection Reagent (1 ml)*	RNAiFect Reagent and buffer, for up to 170 transfections in 24-well plates; up to 500 transfections in 96-well plates	301605
TransMessenger Transfection Reagent (0.5 ml)	For 60 transfections in 6-well plates or 80 transfections in 12-well plates	301525
Lamin A/C siRNA (5 nmol)	5 nmol of siRNA duplex targeted against the human lamin A/C gene	1022050
RNAi Starter Kit	RNAiFect Reagent, siRNA Suspension Buffer, Lamin A/C siRNA, Non-silencing Fluorescein-Labeled Control siRNA	301699
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
QuantiTect Probe RT-PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe RT-PCR Master Mix, 100 µl QuantiTect RT Mix, 2 x 2.0 ml RNase-Free Water	204443
QuantiTect Hs_LMNA Assay (100)	For 100 x 50 µl reactions to detect and quantify human LMNA: 0.5 ml QuantiTect Hs_LMNA Assay Mix (10x)	241338

*Larger sizes available; please inquire.

Easy and rapid establishment of multiplex PCR assays

Holger Engel, Corinna Küppers, and Dirk Löffert

QIAGEN GmbH, Hilden, Germany

Table1. Applications of Multiplex PCR

Source of DNA	Application
Animals/human	Analysis of satellite DNA (e.g., STR analysis or variable number of tandem repeats [VNTR] analysis)
	Typing of transgenic animals
	Lineage analysis, (e.g., of farm animals)
	Detection of pathogens
	Food analysis
	Sex determination
	Detection of mutations
	Amplification of SNP loci
Plants	Analysis of satellite DNA (e.g., STR analysis or variable number of tandem repeats [VNTR] analysis)
	Typing of transgenic plants
	Lineage analysis
	GMO analysis
	Detection of pathogens
Bacteria/viruses	Hygiene analysis
	Detection of pathogens/ diagnostics

Multiplex PCR is a powerful technique that enables amplification of two or more products in parallel in a single reaction tube. It is widely used in genotyping applications and different areas of DNA testing in research, forensic, and diagnostic laboratories (Table 1). DNA tested typically originates from a variety of eukaryotic (human, animal, and plant) and prokaryotic (bacterial and viral) sources. Multiplex PCR is used for screening, typing, and testing wild-type and genetically modified organisms (GMOs), pathogen detection, and for analysis of microsatellite loci, (e.g., in short tandem repeat [STR] analysis). The technique is also increasingly being used in single-nucleotide polymorphism (SNP) analysis, since most SNP identification methods require PCR amplification of the respective genomic loci. Using multiplex PCR, it is possible to analyze multiple genetic markers in a convenient, single reaction procedure that saves both time and materials. If the amount of sample material is limited, multiplex PCR may offer the only possibility to obtain the required information from a given sample. Multiplex PCR is also widely used for screening, typing, and testing wild-type and genetically modified organisms (GMOs), and pathogen detection.

Traditionally, establishing a multiplex PCR assay is a tedious and time-consuming procedure that requires lengthy parallel optimization of multiple parameters, such as concentrations of primers, Mg^{2+} ions, *Taq* DNA polymerase, dNTPs, and additives (1,2). Additionally, buffer composition and cycling parameters frequently need to be optimized, and results may still be dissatisfying.

The QIAGEN® Multiplex PCR Kit is the first kit optimized for multiplex PCR, and provides a ready-to-use 2x master mix that contains preoptimized concentrations of HotStarTaq® DNA Polymerase (which offers a highly-stringent hot-start), $MgCl_2$, and dNTPs. The master mix contains a unique PCR buffer containing the novel synthetic factor MP. Together with optimized salt concentrations, this factor stabilizes specifically bound primers and enables efficient extension of all primers in the reaction (3). By providing default primer concentrations and cycling parameters, tedious and lengthy optimization steps are virtually eliminated. In this article we demonstrate that the QIAGEN Multiplex PCR Kit makes multiplex PCR assays simple and straightforward to establish, independent of DNA source, amount of template, or detection technique used.

Efficient 16-plex PCR over a Range of Template Amounts

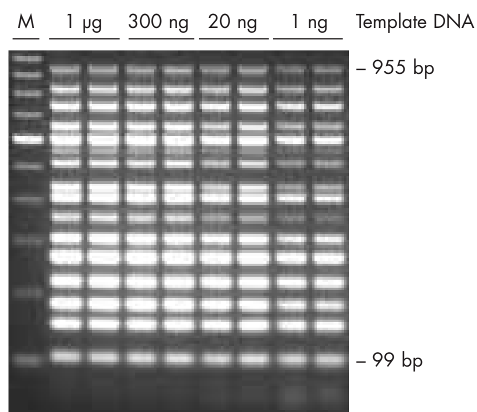


Figure 1 Multiplex PCRs were performed in duplicate using the indicated amount of genomic DNA and 16 pairs of primers that amplified fragments of 99, 150, 181, 222, 269, 310, 363, 414, 446, 523, 564, 610, 662, 756, 845, and 955 bp. **M**: 100 bp ladder.

Materials and methods

Genomic DNA was isolated from human K562 cells using the DNeasy® Tissue Kit. Multiplex PCR using a set of 16 primer pairs specific for different human genomic loci (PCR product sizes: 99–955 bp) was performed for 40 cycles with varying amounts of template DNA using the standard multiplex PCR protocol from the *QIAGEN Multiplex PCR Handbook*. PCR products were analyzed on a 1.6% agarose gel run using 1x TAE buffer (4). Most primers were designed using standard PCR primer-design software or chosen manually; some were taken from the literature. All had a T_m calculated to be ≥60°C using the T_m = 2(AT) + 4(GC) rule.

For STR analysis, genomic and mitochondrial DNA was purified from 200 µl whole blood using the QIAamp® DNA Blood BioRobot® MDx Kit. For multiplex PCR, 1 µl of eluate (approximately 30 ng DNA) was used as template. Multiplex PCR of the amelogenin gene and the STR markers vWA, D7S820, F13A1, HUMTH01, and FES/FPS was performed using the QIAGEN Multiplex PCR Kit and the protocol for amplification of microsatellite loci from the *QIAGEN Multiplex PCR Handbook*. PCR was carried out for 24 cycles using the microsatellite cycling protocol. For visualization of PCR products, one primer from each pair was fluorescently labeled at the 5' end with either 6-FAM™ (vWA, D7S820, F13A1, and amelogenin) or HEX (HUMTH01 and FES/FPS). A 1 µl aliquot of the multiplex PCR product was used for analysis on an ABI PRISM® 377 sequencer.

Results

Sixteen-plex PCR was successfully carried out with varying amounts of genomic DNA as template (Figure 1). Primers were selected to give PCR products of sizes ranging from 99–955 bp that could be well separated by agarose-gel electrophoresis. In this test system, the large size difference between the products presented a difficult challenge for multiplex PCR. Using the standard protocol for multiplex PCR, all products were successfully amplified over the entire range of template DNA amounts tested, from 1 µg down to 1 ng. This range of template amounts covers those typically available to labs performing genotyping of transgenic organisms or detection of pathogens or GMOs. No optimization of reaction chemistry or cycling conditions was required, demonstrating the ease-of-use and robustness of the system.

To show a practical application of multiplex PCR, 5 human STR loci and the amelogenin gene were amplified in a multiplex PCR assay (Figure 2). Fluorescently labeled primers and a DNA sequencer were used to confirm that the respective loci were successfully amplified from human genomic DNA using the QIAGEN Multiplex PCR Kit. Once again, no optimization of the PCR conditions was required. ▶

STR Analysis Using Multiplex PCR

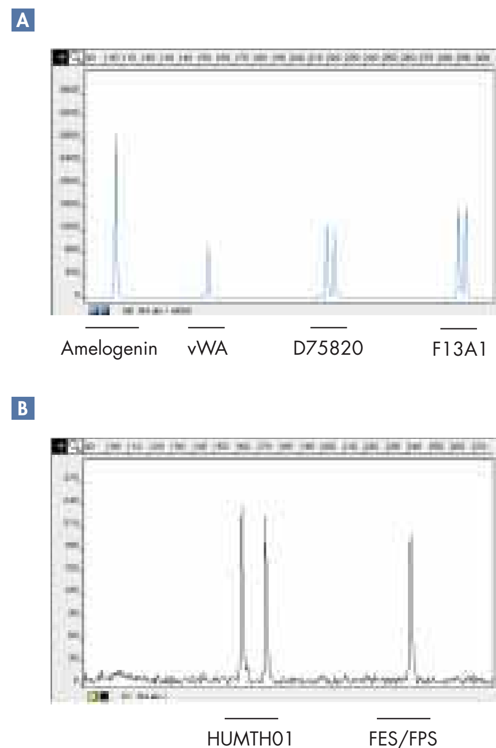


Figure 2 The indicated STR loci were analyzed by multiplex PCR using fluorescently labeled primers. Fluorescent peak traces were obtained using the A FAM or B HEX channel of a DNA sequencer.

Conclusions

- Using the QIAGEN Multiplex PCR Kit enabled every product in a 16-plex PCR to be efficiently amplified over a range of template amounts without any optimization. This is in contrast to traditional multiplex PCR assay development, which is typically a labor-intensive and time-consuming procedure.
- The QIAGEN Multiplex PCR Kit could be used for detection of multiple loci in STR analysis. Once again, no additional optimization was required, demonstrating the robustness of the procedure. Requirements for reagents and samples are minimized.
- As multiplex PCR finds more and more applications in the field of genetic testing, using the QIAGEN Multiplex PCR Kit offers researchers real advantages over those using traditional time- and reagent-consuming methods.

References

1. Henegariu, O., Heerema, N.A., Dlouhy, S.R., Vance, G.H., and Vogt, P.H. (1997) Multiplex PCR: critical parameters and step-by-step protocol. Biotechniques **23**, 504.
2. Multiplex PCR that simply works — the new QIAGEN Multiplex PCR Kit. QIAGEN News 2002, No. 5, 1.
3. Engel, H., Küppers, C., and Löffert, D. (2003) Highly efficient multiplex PCR using novel reaction chemistry. QIAGEN News **2003**, 41.
4. Sambrook, J, Fritsch, E, and Maniatis , T. (1989) *Molecular Cloning : A Laboratory Manual.* 2nd ed. New York: Cold Spring Harbor Laboratory Press.

Ordering Information

Product	Contents	Cat. no.
QIAGEN Multiplex PCR Kit (100)*	For 100 x 50 µl multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix, 5x Q-Solution (1 x 2.0 ml), Distilled Water (2 x 1.7 ml)	206143
"Easy-to-use automation for RNA purification", page 35		
BioRobot EZ1	Robotic workstation for automated purification of nucleic acids using EZ1 kits, installation, 1 year warranty on parts and labor	9000705
EZ1 RNA Cell Mini Kit (48)	For 48 RNA preps: 48 Reagent Cartridges (Cell RNA), Plasticware, RNase-Free Buffers and Reagents	958134
EZ1 RNA Tissue Mini Kit (48)	For 48 RNA preps: 48 Reagent Cartridges (Tissue RNA), Plasticware, RNase-Free Buffers and Reagents	959134
EZ1 RNA Card	Pre-programmed card for BioRobot EZ1 DNA Tissue Protocol	9015590
BioRobot M48	Robotic workstation for automation of magnetic particle technology	9000709
MagAttract RNA Cell Mini M48 Kit (192)	For 192 RNA preps: MagAttract Suspension E, RNase-Free Buffers and Reagents	958336
MagAttract RNA Tissue Mini M48 Kit (192)	For 192 RNA preps: MagAttract Suspension E, RNase-Free Buffers and Reagents	959336

* Bulk size available; please inquire.

High PCR efficiency enables more accurate quantification in real-time PCR

Thorsten Traeger, Miriam Hesse, Andreas Missel, Katharina Machura, and Dirk Löffert

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Differences in individual gene expression levels provide information about changes in cellular metabolism. These changes can be caused by altered environmental or developmental conditions within an organism or by external stimuli (e.g., chemicals). PCR efficiency has a significant effect on the accuracy of calculated gene expression levels. Differences in efficiency can be caused by a number of factors, such as the presence of reverse-transcriptase inhibitors. However, reagents used in real-time RT-PCR can also significantly affect the accuracy of gene expression analysis data. In this article, we demonstrate that optimized reaction chemistries can lead to increased PCR efficiency and to more reliable relative transcript quantification.

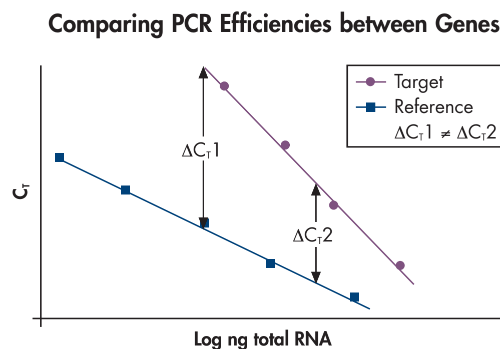


Figure 1 Typical standard curves showing amplification of two targets with different PCR efficiencies.

Impact of PCR efficiency on the accuracy of relative quantification

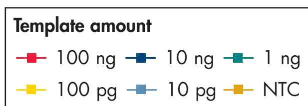
Gene expression levels can be calculated by determining the difference between the amount of a target gene and an endogenous reference gene (e.g., a housekeeping gene, such as β-actin). The difference in threshold cycle (C_T) value, or ΔC_T, between the target and reference gene can be calculated and then directly compared between different samples. This is known as the $\Delta\Delta C_T$ method of quantification (1). However, quantification will only be accurate if both genes are amplified with comparable efficiencies.

To determine whether PCR efficiency is comparable for both genes, standard curves must be generated. If there are differences in PCR efficiency, the resulting standard curves will not be parallel and the difference between C_T values of the target and the reference will not be constant as template amounts are varied (Figure 1). This means that unless this effect is taken into account, the calculated amount of transcript between samples will be inaccurate, leading to false gene expression level data.

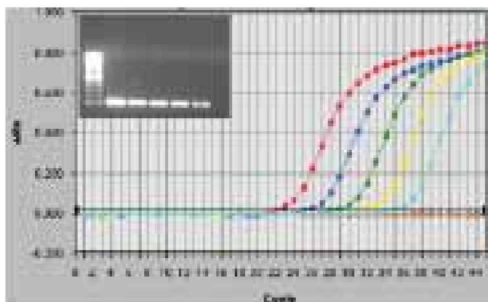
High PCR specificity is a prerequisite for high PCR efficiency

Improved PCR specificity leads to higher PCR efficiency and sensitivity, and a wider dynamic range. This is due to the absence of nonspecific amplification products, which would otherwise compete for PCR reactants during the course of a reaction. High PCR specificity can lead to better efficiency not only when using SYBR® Green I, but also in assays with ▶

Improved PCR Specificity and Efficiency Using Dual-Labeled Probes



QIAGEN



Supplier A_{II}

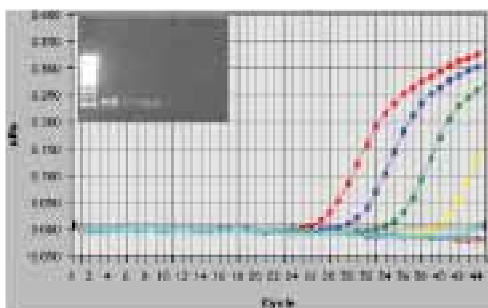


Figure 2 Mouse *Bcl-2* cDNA was amplified using a dilution series of 100 ng to 10 pg cDNA, and gene-specific primers and probe. Reactions were carried out on the ABI PRISM® 7700 Sequence Detection System, using the QuantiTect Probe PCR Kit (**QIAGEN**) or a probe kit (**Supplier A_{II}**). In reactions performed with the QIAGEN® kit, all dilutions were amplified and gave rise to specific products and the C_T difference between each 10-fold dilution was approximately 3. In reactions performed using the kit from Supplier A_{II} this was not the case and the lowest template amount failed to produce a specific PCR product.

dual-labeled probes. Although dual-labeled probes only detect the specific PCR product, competition between the specific PCR product and nonspecific amplification products can still occur. This is shown in Figure 2, where varying yields of PCR product were obtained when using reagents from Supplier A_{II}, indicating co-amplification of undesired background products. This not only leads to reduced sensitivity for lower template amounts and decreased linear range, but also to a PCR efficiency of only 61% (or 89% if the lowest template amount is excluded from the analysis). Furthermore, relative quantification of gene expression levels would be inaccurate. In contrast, the highly specific amplification obtained using the QuantiTect® Probe PCR Kit delivers an amplification efficiency of 98% and also provides a wider dynamic range and superior sensitivity.

How reagent composition affects PCR specificity and efficiency

Optimization of amplification reaction chemistries can influence PCR specificity in two ways; firstly, by preventing formation of nonspecific PCR products at the start of the reaction when temperatures are low, and secondly, by providing highly specific primer annealing during each annealing step. With QuantiTect Probe Kits, specific primer annealing is achieved by using a balanced combination of KCl and $(NH_4)_2SO_4$, which not only stabilizes complementary primer–template binding but also destabilizes nonspecifically bound primers. This destabilization is due to competition of NH_4^+ ions with weak hydrogen bonds between primers and template that form upon nonspecific hybridization. Therefore, with QuantiTect Probe Kits, optimized PCR buffering chemistry enables specific primer binding during each annealing step. In combination with a stringent hot start, provided by HotStarTaq® DNA Polymerase, this ensures highest PCR specificity and leads to very high PCR efficiency (Figure 2). This means that relative transcript quantification is more accurate.

Improving sensitivity of one-step, real-time RT-PCR

It has often been observed that one-step RT-PCR assays show lower sensitivity than two-step RT-PCR assays. When using *Tth* DNA polymerase* for both the reverse transcription step and PCR, this loss of sensitivity is due to the lower efficiency of the reverse-transcriptase activity of the enzyme (2). However, when using reverse transcriptase from retroviral sources, loss of sensitivity is often due to the inhibitory effect of the reverse transcriptase on the PCR (3, 4). This is shown by adding increasing amounts of heat-denatured reverse transcriptase to amplification reactions containing identical amounts of a starting DNA template (Figure 3). The observed shift towards higher C_T values upon addition of increasing amounts of reverse transcriptase demonstrates this strong inhibitory

* An enzyme that catalyzes the polymerization of nucleotides into duplex DNA in the 5' → 3' direction in the presence of magnesium and the polymerization of nucleotides into DNA using an RNA template in the 5' → 3' direction in the presence of manganese.

effect. The QuantiTect Probe RT-PCR Kit contains a reverse-transcriptase–specific additive that negates this inhibitory effect, providing much higher specificity and sensitivity and overcoming this limitation of one-step RT-PCR assay sensitivity (Figure 4).

Conclusions

- Optimized buffering in QuantiTect Probe Kits increases PCR specificity and contributes significantly to higher PCR efficiency, enabling a wider dynamic range in gene expression assays.
- A novel additive for one-step real-time RT-PCR contained in QuantiTect Probe Kits negates the inhibitory effects of reverse transcriptase on PCR, providing robust performance and high real-time RT-PCR sensitivity.

Inhibition of PCR by Reverse Transcriptase

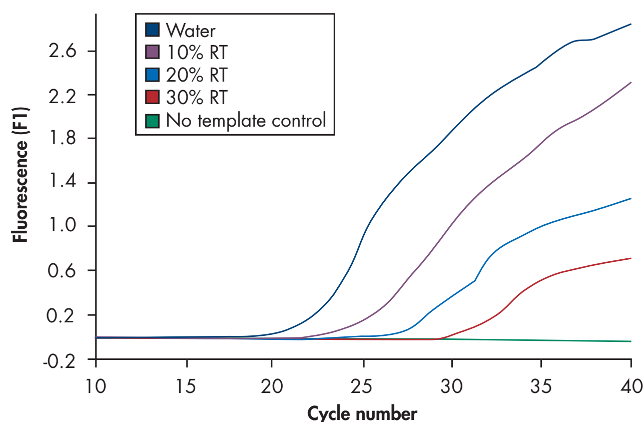


Figure 3 The inhibitory effect of adding heat-denatured reverse transcriptase to PCRs. Amplification reactions were set up containing identical amounts of plasmid DNA and the indicated volume of a heat-denatured RT reaction. Reactions were carried out using standard PCR reagents and a dual-labeled probe. Increasing the proportion of heat-denatured reverse transcriptase in the reaction led to PCR inhibition.

Overcoming RT Inhibition

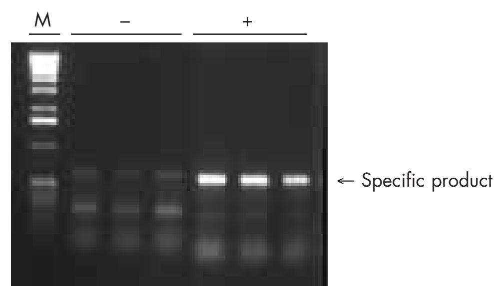


Figure 4 The effect of the proprietary QuantiTect reverse-transcriptase–specific additive on one-step RT-PCR. A 626 bp fragment of the human β-actin gene was amplified using gene-specific primers in one-step RT-PCR. Amplification was performed in standard PCR buffer with Omniscript® Reverse Transcriptase and HotStarTaq DNA Polymerase. Total RNA was used as template (1 ng) and RT-PCR was carried out without (–) and with (+) the reverse-transcriptase–specific additive in the reaction.

References

1. QIAGEN (2004) *Critical Factors for Successful Real-Time PCR.*
2. Cusi, M.G., Valassina, M., and Valensin, P.E. (1994) Comparison of M-MLV reverse transcriptase and *Tth* polymerase activity in RT-PCR of samples with low virus burden. BioTechniques **17**, 1034.
3. Sellner, L.N., Coelen, R.J., and Mackensie, J.S. (1992) Reverse transcriptase inhibits *Taq* polymerase activity. Nucleic Acids Res. **20**, 1487.
4. Chandler, D.P., Wagnon, C.A., and Bolton, H., Jr. (1998) Reverse transcriptase (RT) inhibition of PCR at low concentrations of template and its implications for quantitative RT-PCR. Appl. Environ. Microbiol. **64**, 669.

Related article in this issue

Easy and rapid establishment of multiplex PCR assays (page 46)

Ordering Information

Product	Contents	Cat. no.
QuantiTect Probe PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe PCR Master Mix (providing a final concentration of 4 mM $MgCl_2$), 2 x 2.0 ml RNase-Free Water	204343
QuantiTect Probe RT-PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe RT-PCR Master Mix (providing a final concentration of 4 mM $MgCl_2$), 100 µl QuantiTect RT Mix, 2 x 2.0 ml RNase-Free Water	204443

* Larger kit sizes available; please inquire.

Easy and efficient automated DNA preps from reference and differentially extracted forensic samples

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The BioRobot® EZ1 workstation was used with the EZ1 DNA Tissue Kit and the EZ1 DNA Forensic Card to successfully purify genomic DNA from a wide range of typical forensic casework samples, including blood, saliva, and sexual assault samples. Purification was cross-contamination–free and as efficient as the established, manual extraction method using phenol–chloroform. STR analysis of DNA from sperm cells differentially extracted from mixed samples indicated that purified DNA was of high quality and did not contain PCR inhibitors.

The field of forensic science is highly demanding, requiring efficient and reproducible purification of DNA from a wide variety of sample types with minimal risk of contamination or handling errors. To obtain clearly interpretable results in downstream assays, robust and reliable technologies are required to process the wide range of potentially degraded or limited evidence samples and reference samples that are encountered in forensic casework. The BioRobot EZ1 workstation offers forensic laboratories a fast and convenient solution for DNA purification. EZ1 kits provide an efficient method for the purification of high-quality genomic DNA from a variety of sample types through fully automated magnetic-particle technology. The process gives 1–6 pure DNA samples in as little as 45 minutes.

Materials and methods

For the cross-contamination study, 6 concentrated blood samples were extracted and interspersed alongside 6 blank samples. The order of the samples and blanks was reversed for the second run of the BioRobot EZ1. The concentrated blood samples comprised 100 µl of whole blood in 100 µl distilled water. The blank samples were 200 µl of distilled water. The extractions were carried out on the BioRobot EZ1 in two extraction runs, and the purified DNA was quantified by slot-blotting. For the slot-blot analysis, blank samples were 10 times more concentrated than the blood samples. All slot-blot analyses were detected using a luminol reaction captured with a CCD camera.

To investigate DNA purification from differential extractions, sexual-assault–type samples containing semen mixed with saliva on cotton swabs were processed. The samples were resuspended in 50 µl distilled water before centrifugation for 2–5 minutes at 10,000 x *g* to pellet sperm and any other cells. A preliminary digestion (140 µl digest buffer and 10 µl proteinase K for 1–2 hours) was performed to lyse non-sperm cells. The remaining sperm cells were pelleted by centrifugation, and the supernatant (non-sperm fraction) was removed and saved before a further digestion with larger volumes of digest buffer and proteinase K. The sperm count of the samples was assessed by microscopic examination, and the expected yield of DNA from the sperm fractions were calculated based on the estimate that each sperm cell contains 3 pg of genomic DNA. The sperm fractions, in 50 µl distilled water, were treated with 130 µl digest buffer, 10 µl proteinase K, and 10 µl DTT. The sperm and non-sperm fractions were both purified using the BioRobot EZ1 workstation.

In further experiments, dilutions of dried blood samples were treated with 190 µl digest buffer and 10 µl proteinase K. For saliva samples, after microscopic examinations, which included solubilizing the cells with 50 µl distilled water, dried diluted saliva stains were treated with 140 µl digest buffer and 10 µl proteinase K.

DNA with yields sufficient for further downstream assays were obtained from 5 µl of liquid blood reference samples, 3 x 3 mm cuttings from reference bloodstains on filter paper (Schleicher & Schuell), or approximately a quarter of a reference mouth swab. Reference samples were treated with 190 µl digest buffer and 10 µl proteinase K before BioRobot EZ1 purification (data not shown).

Results and discussion

DNA purified using the BioRobot EZ1 workstation was free from cross-contamination (Figure 1) and provided sufficient DNA from saliva, dried blood, and semen samples for further STR analyses. The DNA yields from the BioRobot EZ1 purification method were found to be equivalent to those obtained using phenol–chloroform extractions followed by a commercial method of DNA concentration (Figure 2).

Comparisons of DNA yields from BioRobot EZ1 extractions of sperm fractions demonstrated a high efficiency over a wide range of sperm counts and was comparable to the phenol–chloroform method (Figure 2C). STR analysis of DNA purified from the sperm fractions using the BioRobot EZ1 showed clear, strong, and easily interpreted profiles without any sign of enzyme inhibition. DNA was also purified from the non-sperm fractions of the sexual-assault–type samples (Figure 3). ▶

Cross-Contamination–Free Purification

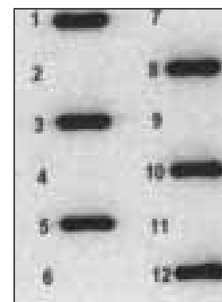

Figure 1 Slot-blot analysis showed no detectable sample-to-sample contamination within or between extraction runs. The single-use reagent cartridges, filter barrier pipet tips, and the linear extraction process all contribute to decreasing the possibilities of cross contamination during DNA purification.

Efficient Automated Purification of DNA from Frequently Encountered Evidence Samples

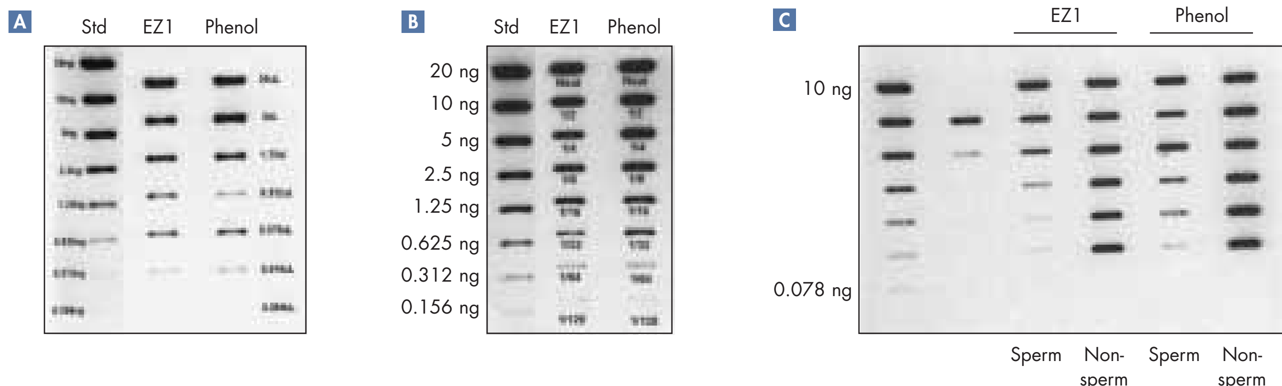

Figure 2 Slot-blot analysis of samples extracted with the BioRobot EZ1 (**EZ1**) and with phenol–chloroform (**Phenol**). **Std**: DNA mass standards. **A** Serial dilutions of blood, from 20 µl to 4 nl. **B** Serial dilutions of saliva stains containing from 50 µl dried neat saliva to 39 nl of saliva placed on fabric. 5 x 5 mm cuttings were used for purification. **C** Dilutions of DNA purified from differentially extracted sperm and non-sperm fractions.

Clean and Full STR Analyses from Sperm and Non-sperm Fractions

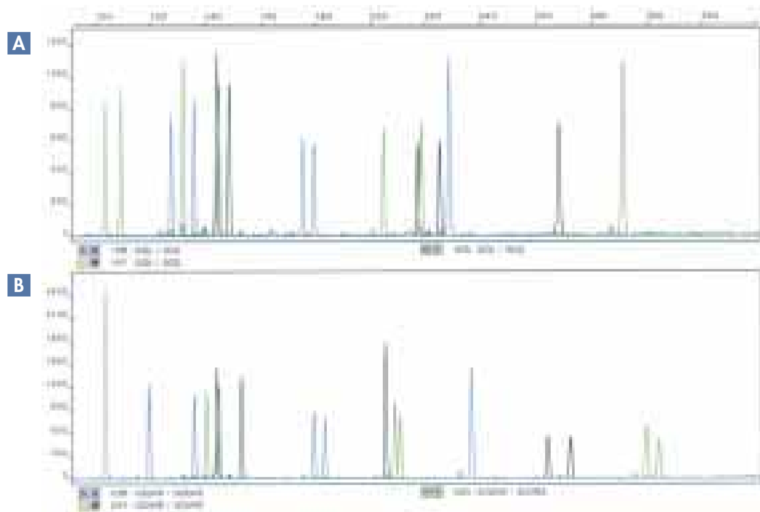

Figure 3 A STR profile from the sperm fraction of a differential extraction purified with the BioRobot EZ1 (a 50 µl elution volume was used for all sperm fraction samples). B STR Profile from the non-sperm fraction of a differential extraction purified with the BioRobot EZ1 (a 200 µl elution volume was used for all non-sperm fraction samples).

Conclusions

The BioRobot EZ1 workstation, together with the EZ1 DNA Tissue Kit and EZ1 DNA Forensic Card, provided:

- Automated DNA purification from a wide range of sample types, including casework samples containing limited amounts of DNA.
- DNA yields comparable to those obtained using organic extraction followed by micro-concentration.
- Purification of cross-contamination–free DNA that performed well in PCR-based techniques such as STR analysis.
- Rapid purification of 1–6 samples in as little as 45 minutes, including pretreatment.

Visit www.qiagen.com/goto/EZforensicDNA today to discover more about easy solutions for forensic sample prep!

Ordering Information

Product	Contents	Cat. no.
BioRobot EZ1	Robotic workstation for automated purification of nucleic acids using EZ1 kits, installation, 1 year warranty on parts and labour	9000705
EZ1 DNA Tissue Kit (48)	48 Reagent Cartridges (Tissue), 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes (2.0 ml), 50 Elution Tubes (1.5 ml), Buffer G2, Proteinase K	953034
EZ1 DNA Forensic Card	Pre-programmed card for BioRobot EZ1 forensic protocols	9015864

CE-certified diagnostic sample preparation

The new range of QIAamp® DSP Kits for diagnostic sample preparation provides the highest standards in safety, quality, and performance according to EU Directive 98/79/EC on in vitro diagnostic (IVD) medical devices. Each kit is intended as a generic sample-preparation system that can be easily integrated into any diagnostic workflow through its compatibility with a broad range of sample-collection devices and assay systems. Two QIAamp DSP Kits are now available. More kits are coming soon.

QIAamp DSP Kits provide:

- **CE-certified product quality** — complying with EU Directive 98/79/EC
- **Easy integration into diagnostic workflows** — compatible with many other products for upstream sample collection and downstream assay
- **Time savings and lower costs** — reduced validation and proven product performance
- **Rapid purification of high-quality nucleic acids** — using established QIAamp silica-membrane technology

A range of kits for diagnostic sample preparation

With the introduction of EU Directive 98/79/EC, all IVD products sold in the countries of the EU must meet the same high standards. Compliance with the directive is certified by labeling the IVD product with the CE mark. QIAGEN has used this directive as an opportunity to continue developing and producing products of the highest standards, and to underline our commitment to in vitro diagnostics. In addition to offering the benefits of complying with the directive (Table 1), QIAamp DSP Kits have a generic character, allowing their use in any diagnostic workflow, regardless of the product used for sample collection and the product used for assay.

QIAamp DSP Kits Fit Easily into Diagnostic Workflows

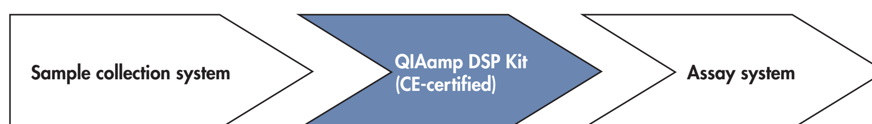

Table 1. Benefits of Compliance of QIAamp DSP Kits with EU Directive 98/79/EC

Benefit	Achieved through:
Certified quality system	Compliance with ISO 9001:2000 / ISO 13485:2003
Greater product safety	Product development according to risk management as defined in ISO 14971
Consistent quality and batch-to-batch homogeneity	Validated manufacturing processes and intensive quality control
Proven product performance and shelf life	Performance studies carried out according to EN 13612 and expiration dates set according to EN 13640
Easy-to-use instructions	Handbook developed according to EN 375 and translated into different languages

QIAamp DSP Kits are based on established QIAamp technology for rapid purification of high-quality nucleic acids. The QIAamp silica membrane specifically binds nucleic acids in a lysed sample, while the rest of the lysate is removed by centrifugation or vacuum pressure. The bound nucleic acids are efficiently washed to remove contaminants before being eluted.

The first 2 QIAamp DSP Kits to be launched are the QIAamp DSP Virus Kit and the QIAamp DSP DNA Blood Mini Kit (see below). Future products will include kits for nucleic acid purification from other sample types as well as automated sample preparation systems.

Purification of viral nucleic acids from serum and plasma

The QIAamp DSP Virus Kit provides rapid purification of viral nucleic acids from human plasma and serum samples for use in sensitive downstream assays. Samples can contain citrate or EDTA, and can be fresh, lyophilized, or frozen.

Using the QIAamp DSP Virus Kit allows purification of any viral nucleic acid, with linear purification and detection over a wide range of titers (see examples in Figure 1). Using the kit also allows sensitive and reproducible detection of nucleic acids in downstream assays (see Table 2 for examples of detection limit [DL]).

Table 2. Sensitive Detection of Viral Nucleic Acids

Assay	Elution volume	95% cut off
artus™ RealArt™ HBV DNA	20 µl	2.31 IU/ml (n=240)
artus RealArt HCV RNA	20 µl	24.31 IU/ml (n=192)
AMPLICOR® HIV RNA	60 µl	90.92 IU/ml (n=209)
TaqMan® HBV DNA	60 µl	4.73 IU/ml (n=192)

Nucleic acids were purified from virus samples and eluted in 20 µl or 60 µl elution buffer. Purified nucleic acids were assayed using a range of assays. Detection limit (DL) was determined according to ICH guidelines. Data from *QIAamp DSP Virus Kit Handbook* (1024585 04/2004).

Nucleic acids are eluted in a low volume of 20 µl or 60 µl, which makes them highly suited for use in any sensitive downstream assay based on amplification or modification of nucleic acids, such as PCR, RT-PCR, and LCR.

Purification of genomic DNA from whole blood

The QIAamp DSP DNA Blood Mini Kit is the world's first CE-certified standalone diagnostic sample preparation system. The kit provides rapid purification of genomic DNA from human whole blood samples for use in any DNA-based downstream assay. The generic character of the kit allows compatibility with blood samples containing the anti-coagulants citrate or EDTA (Table 3). ▶

Linear Yields of Viral Nucleic Acids

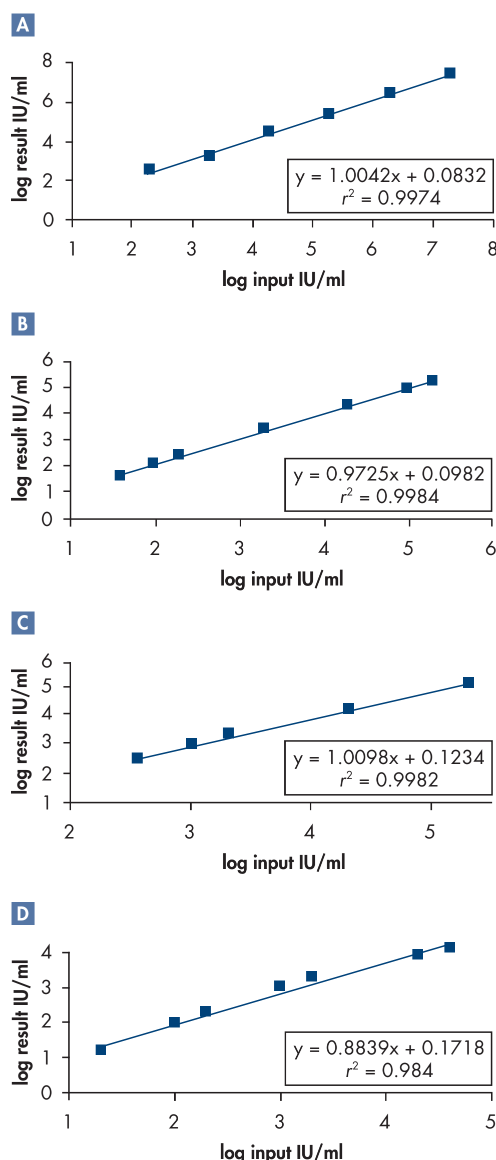

Figure 1 Nucleic acids were purified from virus samples of different titers. Nucleic acids were eluted in 60 µl elution buffer and assayed using TaqMan assays for A HIV RNA and B HBV DNA, and using COBAS® AMPLICOR MONITOR Tests for C HIV RNA and D HBV DNA. Data from *QIAamp DSP Virus Kit Handbook* (1024585 04/2004).

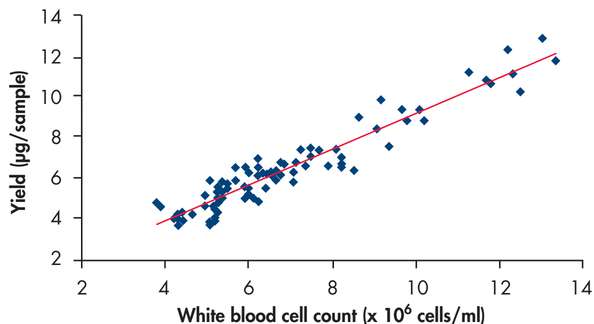

Figure 2 White blood cell counts of healthy donors were determined and were in the range 3.8 x 10^6 – 1.34 x 10^7 cells/ml. DNA was purified from 200 µl blood samples using the QIAamp DSP DNA Blood Mini Kit and eluted in 200 µl elution buffer. Eighty-seven triplicate samples were processed. Linear yields of DNA were obtained in the range of white blood cell counts tested. Data from *QIAamp DSP DNA Blood Mini Kit Handbook* (1025818 01/2004).

Table 3. Blood Collection Using a Range of Different Tubes

Primary tube	Manufacturer	Nominal volume	Average yield from 200 µl
BD Vacutainer™ 9NC	BD	9 ml	6.4 µg
BD Vacutainer K3E	BD	10 ml	6.6 µg
BD Vacutainer K2E	BD	6 ml	6.4 µg
S-Monovette® EDTA	Sarstedt	9 ml	6.5 µg
S-Monovette CPDA1	Sarstedt	8.5 ml	6.3 µg
Vacuette® K3E	Greiner Bio-One	9 ml	6.5 µg
Vacuette 9NC	Greiner Bio-One	2 ml	6.3 µg

For each type of primary tube, blood was collected from 11 donors. For each donor, DNA was purified from 200 µl samples in triplicate using the QIAamp DSP DNA Blood Mini Kit. The table shows average DNA yields from 33 purification procedures. Data from *QIAamp DSP DNA Blood Mini Kit Handbook* (1025818 01/2004).

Linear purification of DNA has been demonstrated for blood samples with white blood cell counts in the range of 3.8 x 10^6 to 1.34 x 10^7 cells/ml (Figure 2). Purified DNA is ready to use in any downstream assay based on amplification or modification of DNA, such as PCR.

Conclusion

CE-certified QIAamp DSP Kits rapidly purify high-quality nucleic acids that are ready to use in any sensitive downstream assay. By complying with EU Directive 98/79/EC and fitting easily into diagnostic workflows, the kits save both time and effort.

Contact us today to discover CE-certified technologies that allow rapid purification of nucleic acids for downstream assays!

Ordering Information

Product	Contents	Cat. no.
QIAamp DSP Virus Kit*	For 50 preps: QIAamp MinElute® Columns, Buffers, Reagents, Tubes, Column Extenders, VacConnectors, and Multi-language Handbook†	60704
QIAamp DSP DNA Blood Mini Kit*	For 50 preps: QIAamp Mini Spin Columns, Buffers, Reagents, Tubes, VacConnectors, and Multi-language Handbook†	61104

* Kit is currently available in Austria, Belgium, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, the Netherlands, Norway, Portugal, Spain, Switzerland, and the United Kingdom.

† Handbook is currently available in English, French, German, Greek, Italian, Portuguese, and Spanish.

RNA purification

Why do I get strange peaks sometimes in Agilent bioanalyzer electropherograms?

When analyzing RNA on the Agilent 2100 Bioanalyzer, you may sometimes notice a strange peak, or it may not be possible to clearly distinguish the ribosomal RNA peaks (see Figure 1 A and B). This could be due to DNA contamination.

Large amounts of DNA can obscure the ribosomal peaks. We recommend using the QIAGEN® RNase-Free DNase Set (cat. no. 79254) for easy and efficient on-column DNase digestion during RNA purification with RNeasy®, PAXgene™, and QIAamp® Kits. The RNase-Free DNase Set is also a standard component of the RNeasy Fibrous Tissue Kits (cat. nos. 74704 and 75742) and the RNeasy Micro Kit (cat. no. 74004).

Examples of Total RNA with and without DNA Contamination

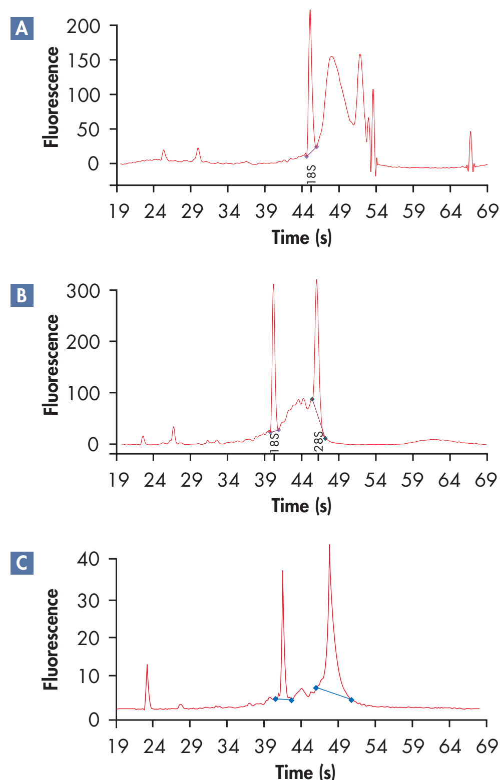

Figure 1 Total RNA was analyzed on the Agilent 2100 Bioanalyzer. A and B Examples of total RNA with genomic DNA contamination. C High-quality RNA isolated using the RNeasy Micro Kit, with the integrated on-column DNase digest.

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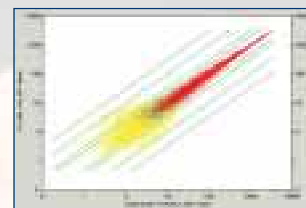
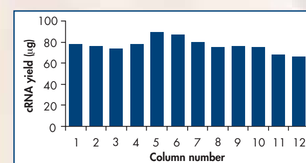


Good correlation with manual target preparation procedure



Average yield of cRNA from 96 RNA samples from HeLa S3 cells

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