

### For fully automated, high-throughput purification of cellular RNA from blood stabilized in PAXgene Blood RNA Tubes

The PAXgene Blood RNA MDx Kit\* is for fully automated, high-throughput purification of cellular RNA on the BioRobot® MDx or BioRobot Universal System. Used with PAXgene Blood RNA Tubes for sample collection and stabilization, the system provides a complete preanalytical solution for collection and stabilization through to purification of high-quality RNA to support high-throughput projects, such as clinical trials or other large research studies.

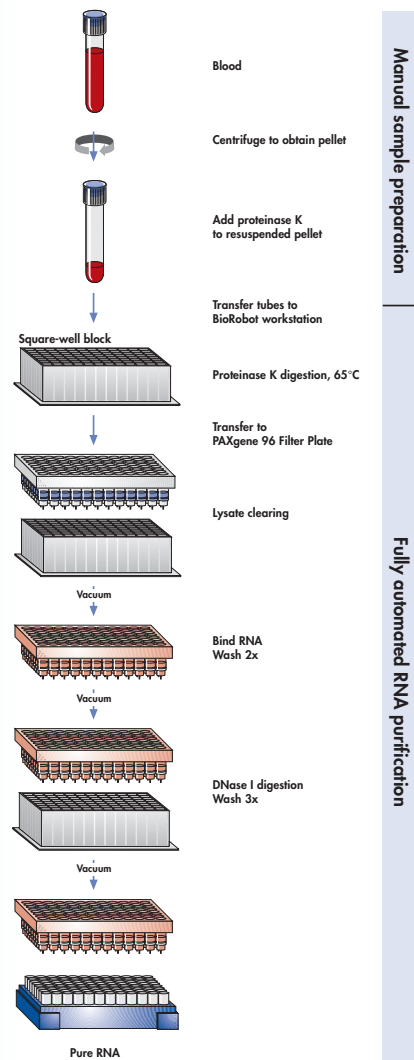
### Benefits of the PAXgene Blood RNA MDx system:

- **Instant stabilization of RNA at point of collection** — integrated system for use with PAXgene Blood RNA Tubes
- **Optimized purification of high-quality RNA** — standardized, automated processing of 48 or 96 samples in parallel
- **Fully automated RNA purification procedure** — on the BioRobot MDx or BioRobot Universal System

### Integrated system for collection, stabilization, and automated high-throughput purification

Blood samples (2.5 ml) are collected in PAXgene Blood RNA Tubes, and may be stored or transported at room temperature if desired (Müller, M. C. et al [2002] Leukemia **16**, 2395). The simple purification procedure begins with a centrifugation step to pellet the contents of each PAXgene

### The PAXgene Blood RNA Procedure



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Blood RNA Tube. Proteinase K and an optimized incubation buffer are added to digest proteins. The tubes are then transferred to the BioRobot MDx or BioRobot Universal System.

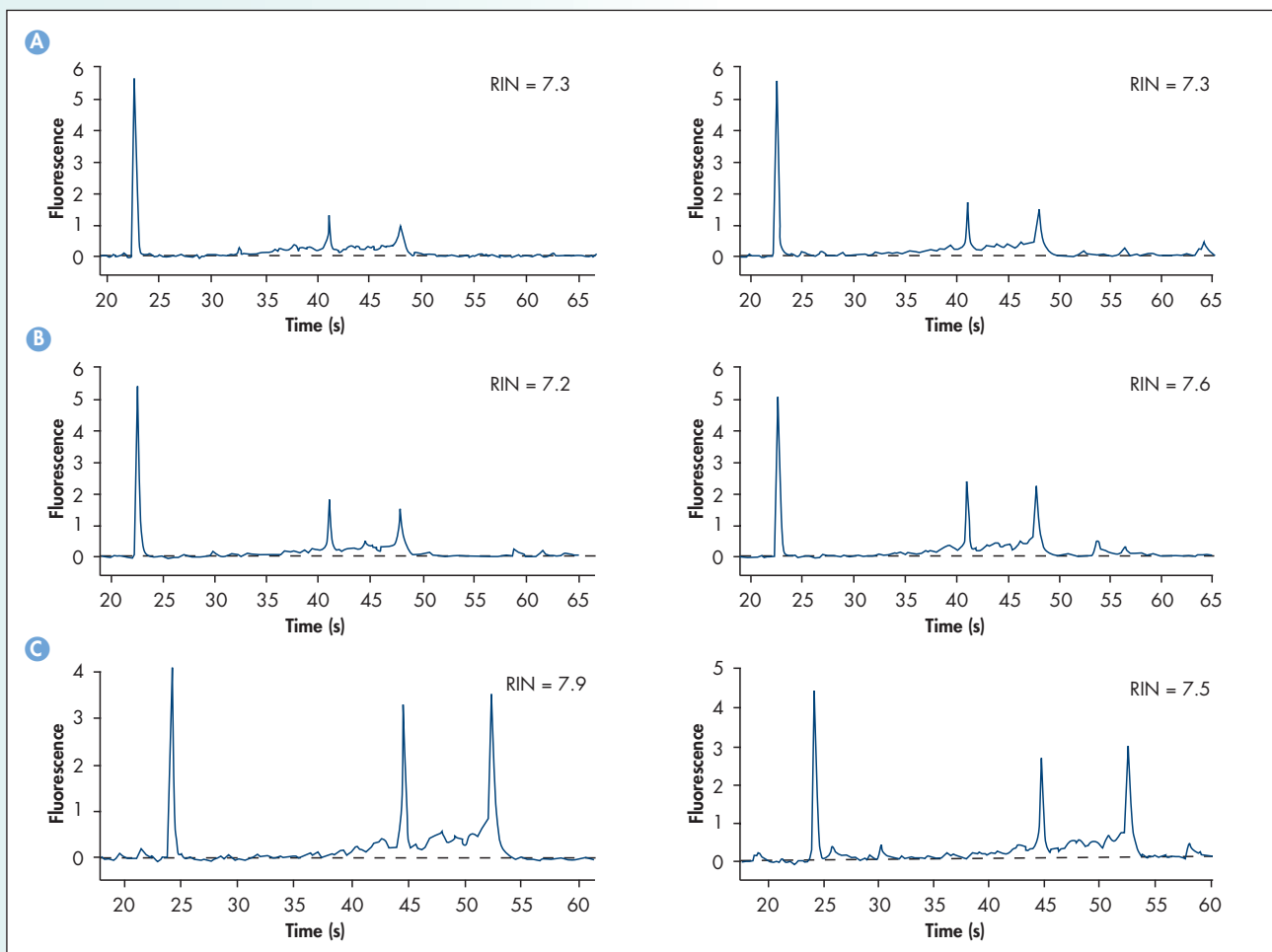
After setup of buffers and plasticware, guided by the QIAsoft Operating System, the BioRobot workstation carries out the fully automated RNA purification procedure (see flowchart, page 1). Following purification, a final heat treatment of the eluate enhances performance in downstream applications. Total processing time is 3–4 hours, with a maximum of 25–30 minutes of hands-on time. Protocols are available for batches of 48 or 96 samples per run.

### High-quality RNA with efficient DNase digestion

The automated PAXgene Blood RNA procedure provides high-quality RNA with high intra-donor repeatability (Figure 1). Since yields are highly donor-dependent, individual yields may vary.

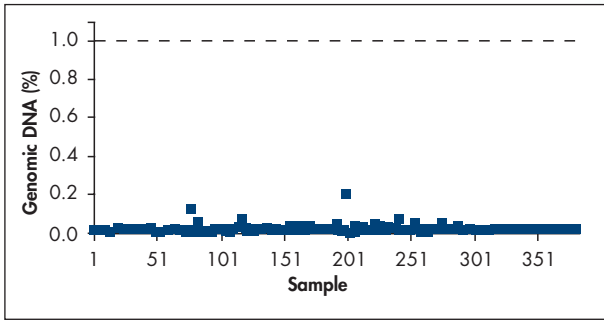
An integrated DNase treatment ensures that genomic DNA contamination is minimized, typically to <0.1% (Figure 2). The robust and standardized procedure is designed to minimize the risk of sample-to-sample cross-contamination (Figure 3).

### High-Quality RNA from Different Donors



**Figure 1** Blood was collected in duplicate from 3 donors into PAXgene Blood RNA Tubes. RNA was purified using the PAXgene Blood RNA MDx Kit on the BioRobot MDx workstation. RNA was analyzed with an Agilent® 2100 bioanalyzer. The RNA integrity number (RIN), indicated on each scan, shows the high quality of the purified RNA. **A** Analysis of 2 samples from donor A. **B** Analysis of 2 samples from donor B. **C** Analysis of 2 samples from donor C.

### Very Low Levels of Genomic DNA



**Figure 2** RNA was purified from 384 blood samples collected in PAXgene Blood RNA Tubes and processed in 4 runs on the BioRobot MDx using the PAXgene Blood RNA MDx Kit. Genomic DNA was quantified using a genomic-DNA-specific real-time TaqMan® PCR assay with primers and probe for the  $\beta$ -actin gene. The amount of DNA present is given as a percentage of the total nucleic acid content.

### High yields of RNA for all downstream applications

The fully automated PAXgene Blood RNA procedure provides RNA yields comparable to the manual PAXgene 96 Blood RNA procedure (typically  $\geq 80\%$ ). Typical yields of RNA isolated from 2.5 ml healthy, human, whole blood ( $4.6 \times 10^6 - 1.1 \times 10^7$  leukocytes/ml) are  $\geq 3 \mu\text{g}$  for  $>95\%$  of the samples processed.

Purified RNA is ready to use in downstream applications, such as quantitative, real-time RT-PCR, with no RT-PCR inhibition detected (Figure 4). Intersystem variation is minimal and well within the error range of typical real-time RT-PCR assays (Figure 5).

### No Detectable Cross-Contamination

	1	2	3	4	5	6	7	8	9	10	11	12
A	28.8	X	28.1	X	27.8	X	28.0	X	28.7	X	30.6	X
B	X	28.7	X	27.3	X	28.8	X	27.9	X	29.0	X	31.2
C	28.6	X	27.7	X	28.0	X	28.8	X	28.0	X	30.2	X
D	X	28.4	X	27.9	X	29.2	X	28.6	X	28.4	X	31.0
E	28.0	X	28.2	X	27.5	X	28.6	X	28.0	X	29.9	X
F	X	29.0	X	27.5	X	27.5	X	28.3	X	27.9	X	32.7
G	28.6	X	27.9	X	28.1	X	27.4	X	27.6	X	30.6	X
H	X	28.2	X	27.9	X	28.0	X	28.0	X	29.2	X	31.3

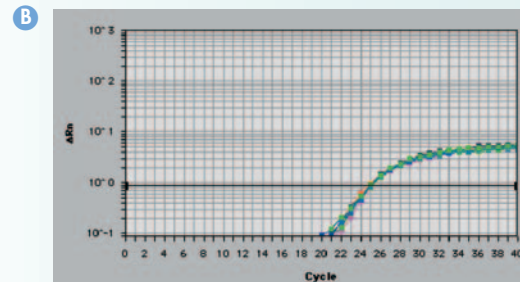
Positive

Negative

**Figure 3** Total RNA was purified from 48 blood samples collected in PAXgene Blood RNA Tubes and 48 water samples, arranged in a checkerboard pattern. RNA purification was carried out using the PAXgene Blood RNA MDx Kit on the BioRobot MDx workstation. All samples were analyzed by quantitative, real-time RT-PCR using primers and probe specific for the 18S rRNA, for 40 PCR cycles. Threshold cycle values ( $C_T$ ) are indicated. X: No RNA was detected in water samples after 40 PCR cycles

### No RT-PCR Inhibition Detected

Proportion of eluate in assay	$C_T$ value	$\Delta C_T$
40%	$20.86 \pm 0.30$	-0.41
30%	$20.52 \pm 0.26$	-0.08
20%	$20.51 \pm 0.18$	-0.07
10%	$20.61 \pm 0.13$	-0.17
0%	$20.44 \pm 0.09$	0.00



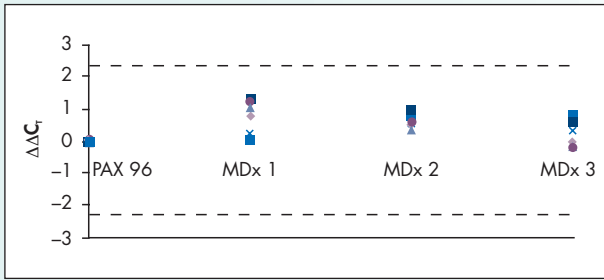
**Figure 4** A total of 48 water samples were processed on the BioRobot MDx workstation using the PAXgene Blood RNA MDx Kit. The eluates were pooled and added to quantitative, real-time RT-PCR assays at the percentage indicated. Each assay was performed in quadruplicate using the same amount of RNA from HeLa cells, with primers and probe for  $\beta$ -actin. **A**  $C_T$  values are given (mean  $\pm$  standard deviation), with the difference between the mean  $C_T$  values and the mean  $C_T$  value for the 0% reactions ( $\Delta C_T$ ). None of the reactions were inhibited ( $\Delta C_T \geq 0.5$ ). **B** Amplification curves for all 20 assays show no detectable inhibition of RT-PCR.

### Applications

The purified RNA is ready for use in a wide range of downstream applications, including:

- High-throughput, quantitative, real-time RT-PCR
- Expression-array and expression-chip analysis
- cDNA synthesis
- RNase and S1 nuclease protection
- Northern, dot, and slot blot analysis
- Primer extension

## High Intersystem Reproducibility



**Figure 5** Blood was collected in fivefold replicates from 6 donors into PAXgene Blood RNA Tubes. RNA was purified using the PAXgene Blood RNA MDx Kit on 3 different BioRobot MDx workstations (**MDx 1, MDx 2, MDx 3**) and manually using the PAXgene 96 Blood RNA Kit (**PAX 96**). Purified RNA was analyzed using quantitative, real-time RT-PCR, with primers and probe for the c-fos transcript. All values were well within the  $\pm 3x$  total precision of the assay (2.34  $C_t$ ), indicated by the horizontal lines.

## Ordering Information

Product	Contents	Cat. no.
PAXgene Blood RNA MDx Kit (4)	For 4 x 96 RNA preps on the BioRobot MDx workstation: 4 PAXgene 96 RNA Plates, 4 PAXgene 96 Filter Plates, Buffers,* Proteinase K, RNase-Free DNase Set, Plasticware, Collection Vessels. To be used with PAXgene Blood RNA Tubes	762431
PAXgene Blood RNA Tubes (100)	100 Blood Collection Tubes. To be used with the PAXgene Blood RNA MDx Kit	762165
BioRobot MDx	Robotic workstation, computer-controlled vacuum pump, computer, QIAsoft MDx Operating System, laboratory cabinet, accessory cabinet, installation, 1-year warranty on parts and labor	900600
BioRobot Universal System	Robotic workstation, computer-controlled vacuum pump, computer, QIAsoft 5 Operating System, installation, 1-year warranty on parts and labor	9001094

\* Wash buffers are labeled with bar codes.

## Discover how you can benefit from stabilization and fully automated RNA purification at [www.qiagen.com/goto/PAXgeneMDx](http://www.qiagen.com/goto/PAXgeneMDx) !

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