

### For storage and transport of bone marrow samples and stabilization and purification of intracellular RNA

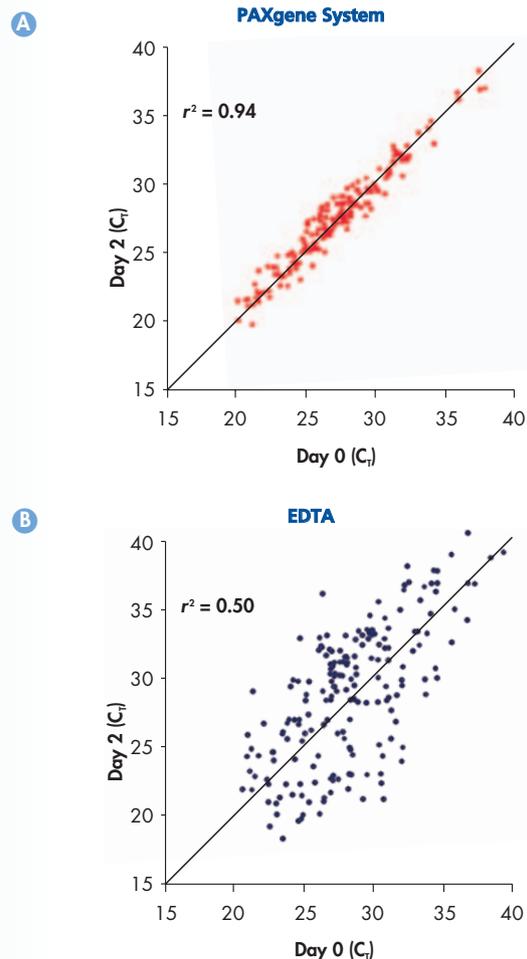
The PAXgene Bone Marrow RNA System consists of PAXgene Bone Marrow RNA Tubes for stabilization, storage, and transportation of human bone marrow samples, and the PAXgene Bone Marrow RNA Kit, for silica-membrane-based RNA isolation and purification in a spin-column format. The system provides a complete preanalytical solution from on-site stabilization and storage through to rapid and efficient purification of high-quality RNA for research applications.\*

### Benefits of the PAXgene Bone Marrow RNA System include

- **Integrated system** — for storage, stabilization, and purification of intracellular RNA from bone marrow
- **Immediate stabilization of intracellular RNA at collection point** — providing in vivo snapshots of gene expression profiles
- **Rapid purification of high-quality intracellular RNA from heterogeneous bone marrow samples** — including cell-rich, viscous samples
- **Safe and easy transportation** — no need for dry ice or liquid nitrogen

\* For Research Use Only. Not for use in diagnostics procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

### RNA Stability in Bone Marrow Samples at Room Temperature



**Figure 1** Bone marrow samples were collected from 39 individuals and immediately transferred to **A** PAXgene Bone Marrow RNA Tubes or **B** EDTA tubes. RNA was purified using the PAXgene Bone Marrow RNA Kit or a reference silica-membrane-based method at day 0 or after 2 days storage at room temperature. Expression levels of 5 marker transcripts for each sample (IL-8, p53, cox-2, IL-1 $\beta$ , c-fos) were quantified using real-time RT-PCR. The high correlation of  $C_T$  values (threshold cycles) for the samples stabilized in PAXgene Bone Marrow RNA Tubes indicates that the levels of these transcripts did not significantly change during storage, in contrast to the samples stored in EDTA tubes.

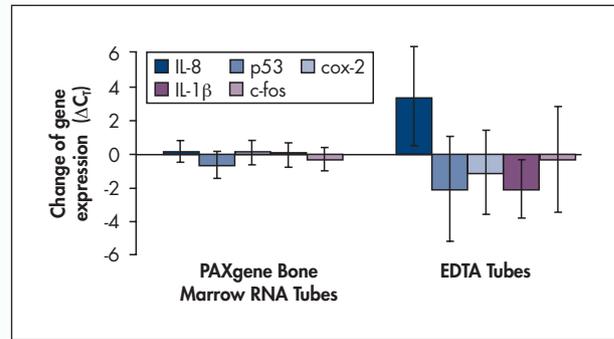
## Integrated system for storage, stabilization, and purification

Bone marrow samples are collected using standard medical practices, with commonly used anticoagulants. A 2 ml sample is then transferred into a PAXgene Bone Marrow RNA Tube, which immediately stabilizes the RNA in the sample for storage at room temperature, refrigerated, or frozen. The samples can be safely transported in the PAXgene Bone Marrow RNA Tube, with no need for dry ice or liquid nitrogen. RNA can be purified days later using the PAXgene Bone Marrow RNA Kit (Figure 1).

## Stabilization of the cellular RNA profile

Copy numbers of individual mRNA species in bone marrow can change significantly during storage or transport at room temperature, making reliable studies of gene expression impossible. PAXgene Bone Marrow RNA Tubes protect RNA molecules from degradation by RNases and minimize *ex vivo* changes in gene expression (Figure 2), enabling accurate analysis of gene expression.

## Stability of 5 Transcripts in Bone Marrow Samples at Room Temperature

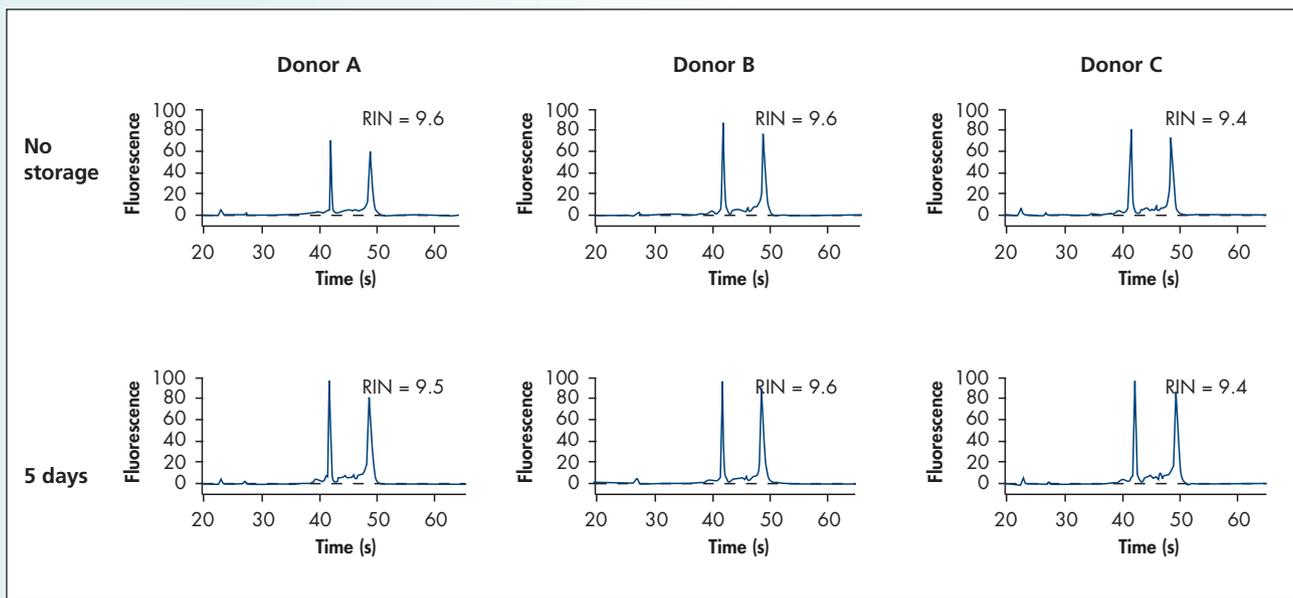


**Figure 2** The changes in mRNA levels ( $\Delta C_t$ ) from day 0 to day 2 for the samples in Figure 1 were calculated for each transcript quantified. The minimal changes and small standard deviation for samples stabilized in PAXgene Bone Marrow RNA Tubes contrasts with the significant changes for samples stored in EDTA tubes.

## Rapid purification of high-quality RNA

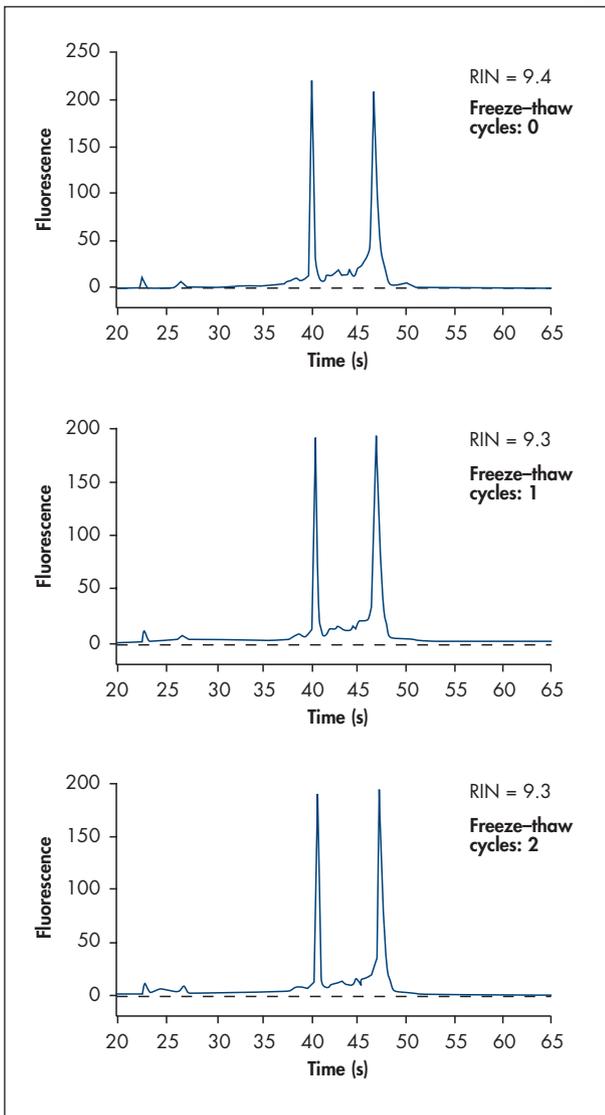
The PAXgene Bone Marrow RNA Kit provides an easy and rapid procedure for isolation and purification of intracellular RNA from human whole bone marrow samples stabilized in PAXgene Bone Marrow RNA Tubes. The kit uses silica-membrane technology in a spin-column format for efficient purification of intact, high-quality

## RNA Stabilized During Storage at 2–8°C



**Figure 3** Bone marrow samples were collected in duplicate and stabilized in PAXgene Bone Marrow RNA Tubes. RNA was purified from the samples using the PAXgene Bone Marrow RNA Kit with no storage or after 5 days storage at 2–8°C. RNA integrity was evaluated using the RNA integrity numbers (RIN), measured on the Agilent® 2100 bioanalyzer. Results are shown for 3 representative donors. The RIN did not change significantly during storage, indicating that the RNA remains stable in the sample.

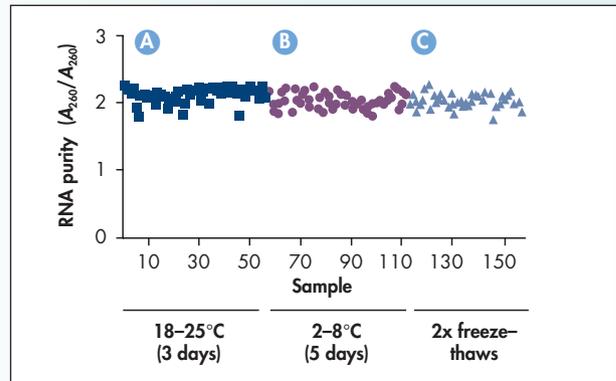
## High RNA Integrity after Freeze–Thaw Cycles



**Figure 4** Bone marrow samples were stabilized in PAXgene Bone Marrow RNA Tubes, and RNA was purified using the PAXgene Bone Marrow RNA Kit after the indicated number of freeze–thaw cycles. The RIN values did not change significantly, indicating that RNA remained intact in the samples up to 2 freeze–thaw cycles.

RNA (Figures 3 and 4). An integrated DNase treatment ensures that genomic DNA is minimized. In-house and external studies showed that genomic DNA was <0.85% in 95% of the samples tested (n = 157).  $A_{260}/A_{280}$  values are typically between 1.8 and 2.2, indicating the high purity of the RNA (Figure 5).

## RNA Purity for Samples Stored under Different Conditions



**Figure 5** Bone marrow samples were stored in PAXgene Bone Marrow RNA Tubes **A** for 3 days at 18–25°C, **B** for 5 days at 2–8°C, or **C** subjected to 2 freeze–thaw cycles. RNA was purified using the PAXgene Bone Marrow RNA Kit, and RNA purity was analyzed by absorbance. The  $A_{260}/A_{280}$  ratios indicate highly pure RNA from samples stored under different conditions.

## Efficient RNA purification from heterogeneous samples

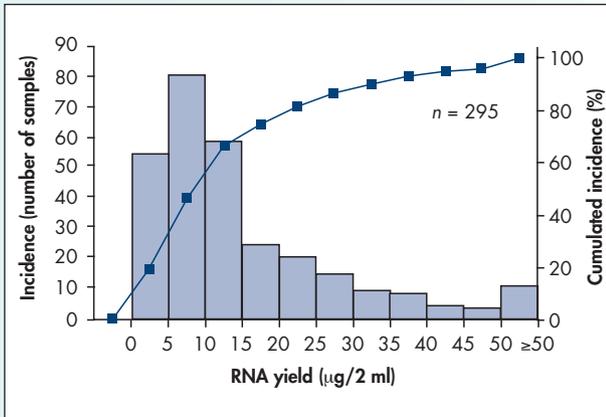
Bone marrow samples are extremely heterogeneous, consisting of varying amounts of cells, tissue, and solid matter (1). RNA yields are highly donor-dependent and can vary greatly from sample to sample as well as between replicates from the same donor. The PAXgene Bone Marrow RNA Kit enables efficient isolation and purification of intracellular RNA from heterogeneous human whole bone marrow samples (Figure 6), including an optimized procedure for cell-rich, viscous samples.

## Applications

RNA purified using the PAXgene Bone Marrow RNA Kit is ready for use in a wide range of downstream applications, including

- Real-time RT-PCR analysis of specific transcripts
- Array analysis of complex expression profiles

## RNA Yields from Bone Marrow Samples



**Figure 6** Bone marrow samples were stabilized in PAXgene Bone Marrow RNA Tubes, and RNA was purified using the PAXgene Bone Marrow RNA Kit. Yields are indicated for 295 samples, from both in-house and external studies. The median yield for the 295 samples was 11.0 µg RNA per 2 ml sample. Note that RNA yields can vary greatly due to the extreme heterogeneity of bone marrow samples.

### Reference

1. Batinic, D. et al. (1990) Relationship between differing volumes of bone marrow aspirates and their cellular composition. *Bone Marrow Transplant.* **6**, 103.

## Ordering Information

| Product                            | Contents   | Cat. no. |
|------------------------------------|--|----------|
| PAXgene Bone Marrow RNA Kit (30)   | For 30 RNA preps: 30 PAXgene Spin Columns, 30 PAXgene Shredder Spin Columns, Processing Tubes, RNase-Free DNase I, RNase-Free Reagents and Buffers. To be used in conjunction with PAXgene Bone Marrow RNA Tubes | 764133   |
| PAXgene Bone Marrow RNA Tubes (50) | 50 Bone Marrow Collection Tubes. To be used in conjunction with the PAXgene Bone Marrow RNA Kit  | 764114   |

**Discover how you can benefit from RNA stabilization and purification from human whole bone marrow at [www.qiagen.com/goto/PAXgeneBM](http://www.qiagen.com/goto/PAXgeneBM) !**

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