PreAnalytiX
A QIAGEN / BD Company

Technical Note
PAXgene® Tissue System

Simultaneous preservation of RNA and morphology in tissue samples fixed with PAXgene Tissue Fix and stored for up to 2 years in PAXgene Tissue Stabilizer at –20 or –80°C

Study Design

Rat (*Rattus norvegicus*) tissue from liver, kidney, spleen, intestine, and lung was divided into samples of approximately 4 x 10 x 10 mm. Samples were placed into standard histocassettes for fixation (3 hours) with PAXgene Tissue Fix and then transferred into a suitable container filled with PAXgene Tissue Stabilizer for long-term storage at –20°C and –80°C. At different time points, samples were removed and directly placed into a tissue processor for processing and paraffin embedding, resulting in PAXgene Tissue fixed, paraffin-embedded (PFPE) tissues (Figure 1).

For analysis of morphology, 4 µm sections from PFPE tissues were stained with hematoxylin and eosin (H&E). RNA was purified from another 4 sections of PFPE tissues (10 µm thick) using the PAXgene Tissue RNA Kit. Reference samples (4 x 10 x 10 mm) from all types of tissue were also snap-frozen in liquid nitrogen (LN2). RNA was purified from 10 mg of each snap-frozen tissue sample using the RNeasy® Mini Kit (QIAGEN) and stored at –20°C until use.

RNA yield was analyzed by measuring the absorbance at 260 nm. RNA integrity was analyzed on an Agilent® Bioanalyzer with the Agilent RNA 6000 Nano assay. End-point one-step RT-PCR was performed with primers specific for a 1065 bp sequence of the rat hypoxanthine phosphoribosyl transferase (HPRT) mRNA. Performance in real-time RT-PCR was analyzed with a TaqMan® Primer/Probe assay for β-actin.

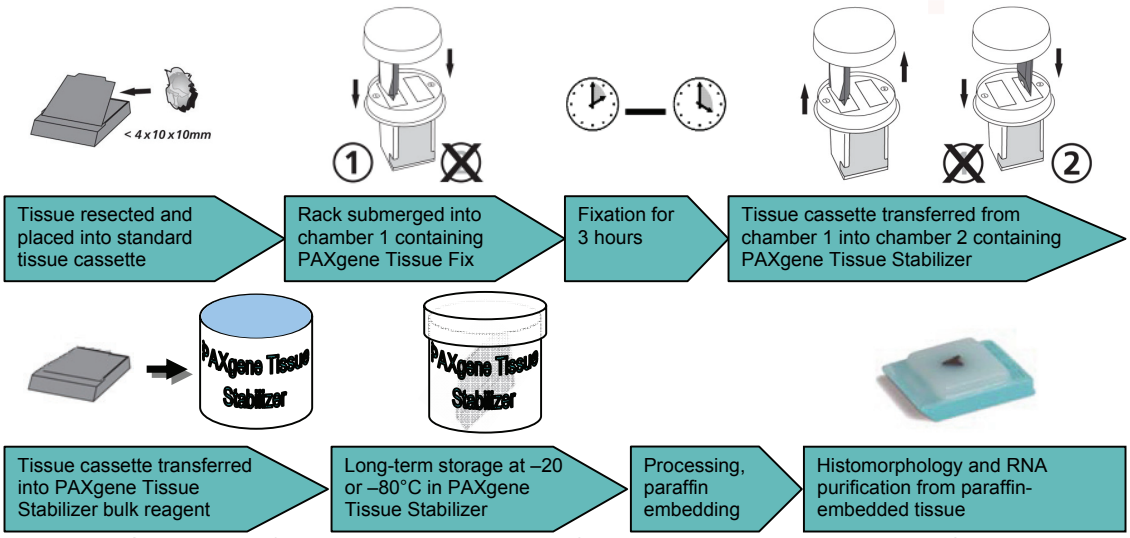

Figure 1. Study workflow. Rat tissue samples fixed with the PAXgene Tissue Container were stored in a container filled with PAXgene Tissue Stabilizer at –20°C or –80°C. At different time points, samples were removed, processed, and paraffin embedded (PFPE tissue).

Results

For specimens stored in the PAXgene Tissue Stabilizer, the long freezing period prior to processing (Figure 2) had no adverse effects on PFPE tissue morphology. The morphology of H&E stained sections was intact and without artifacts after 26 months of storage in the PAXgene Tissue Stabilizer at –20°C or 22 months at –80°C.

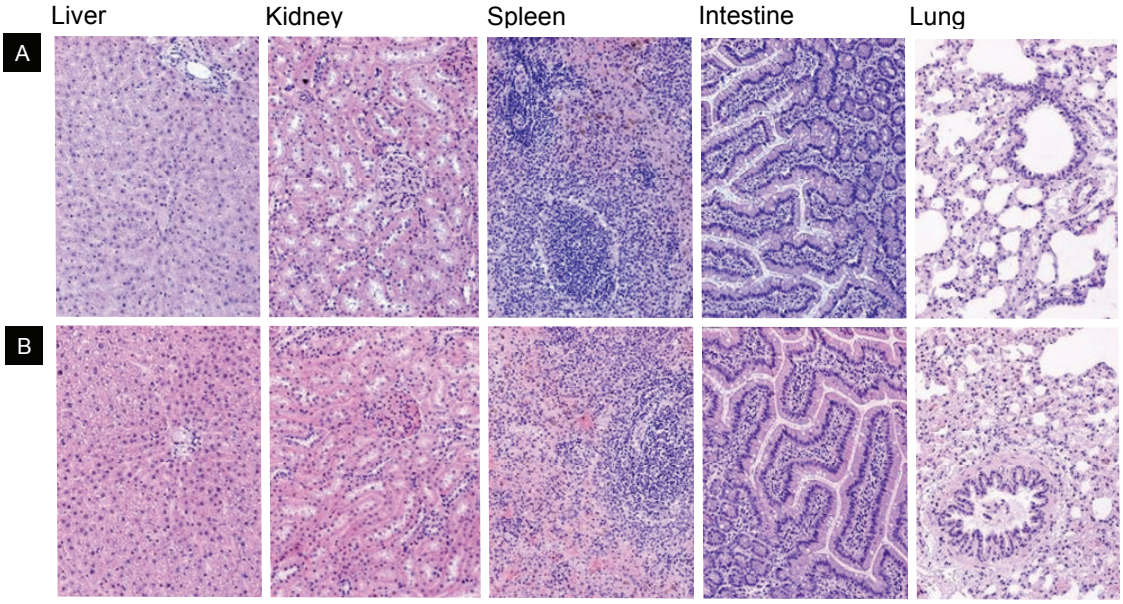

Figure 2. Preservation of morphology. Hematoxylin and eosin staining of 4 µm sections of rat PFPE liver, kidney, spleen, intestine and lung tissue. Tissue was stored prior to processing for **A** 26 months at –20°C or **B** for 22 months at –80°C in PAXgene Tissue Stabilizer. Original magnification 100x.

RNA was isolated in triplicate from PFPE tissue using the PAXgene Tissue RNA Kit. RNA yield varied according to tissue type, from 0.5 µg for lung and up to 10 µg for intestine. Because of the heterogeneous sample sizes, some variation of yield was observed (Figure 3). The RNA integrity number (RIN) was consistently above 6 with the exception of kidney, for which RINs varied between 5.2 and 6.8 (Figure 4). However, there was no correlation between storage time and RNA yield or integrity (Figures 3 and 4).

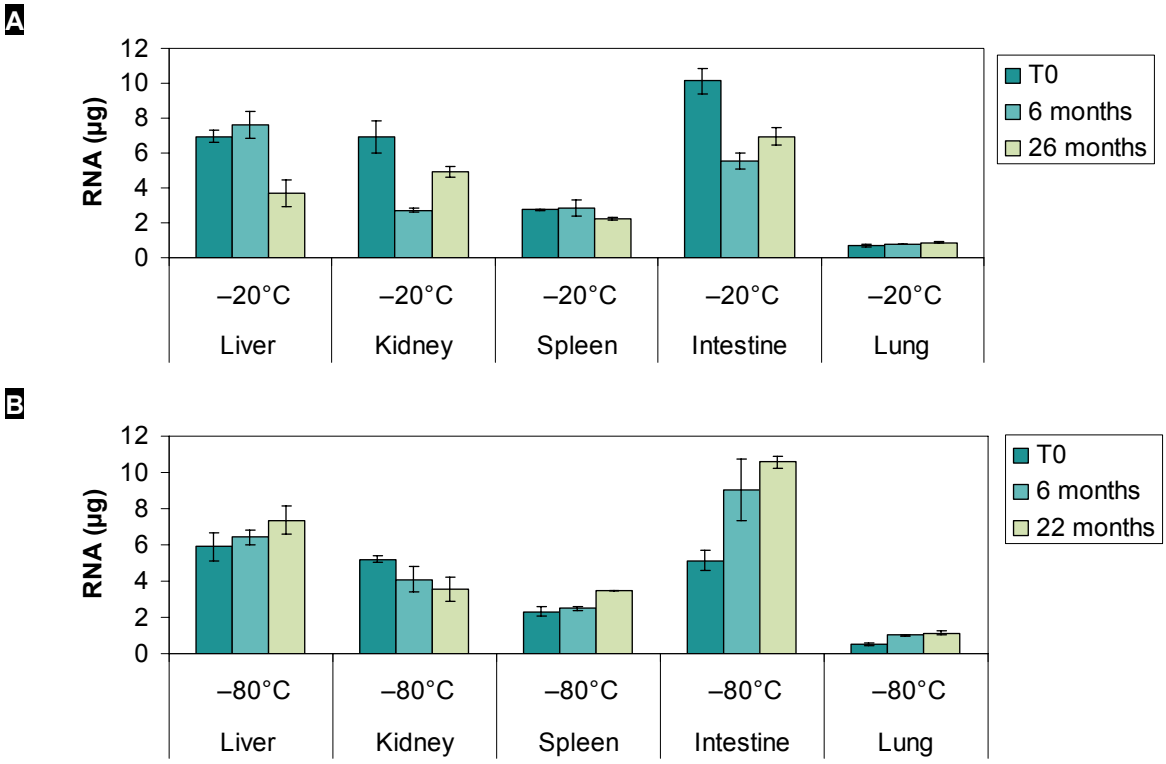

Figure 3. RNA yield from 4 x 10 µm sections of PFPE tissue. RNA yield was analyzed by spectrophotometry using a NanoDrop® instrument. RNA was isolated from PFPE tissue stored prior to processing **A** for up to 26 months at –20°C or **B** for up to 22 months at –80°C. **T0**: Time-zero reference PFPE samples directly processed without storage.

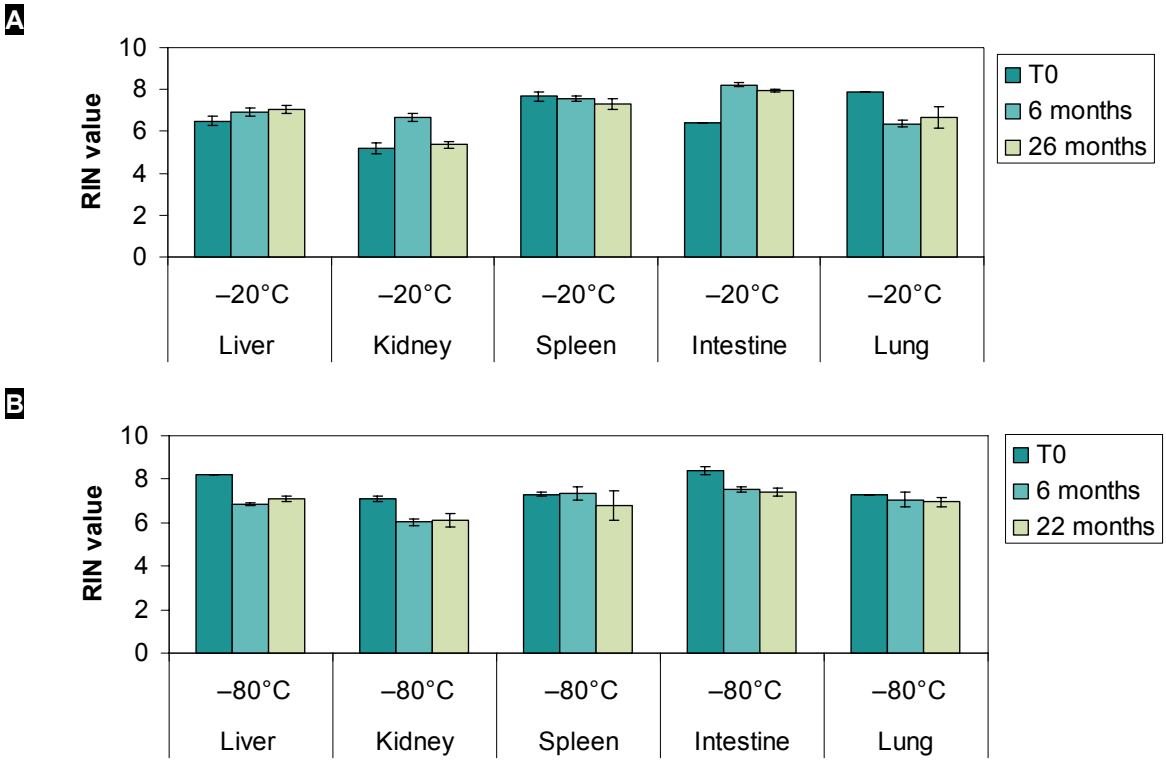

Figure 4. RNA integrity. RNA integrity was analyzed with an Agilent Bioanalyzer to give the RNA integrity number (RIN value). RNA was isolated from PFPE tissue stored prior to processing **A** for up to 26 months at –20°C or **B** for up to 22 months at –80°C. **T0**: Time-zero reference PFPE samples directly processed without storage.

In end-point RT-PCR using 10 ng of RNA, a 1065 bp fragment of the rat HPRT gene could be successfully amplified from all RNA preps from PFPE tissue, regardless of the storage time and temperature of the tissue sample stored in PAXgene Tissue Stabilizer prior to processing (Figure 5).

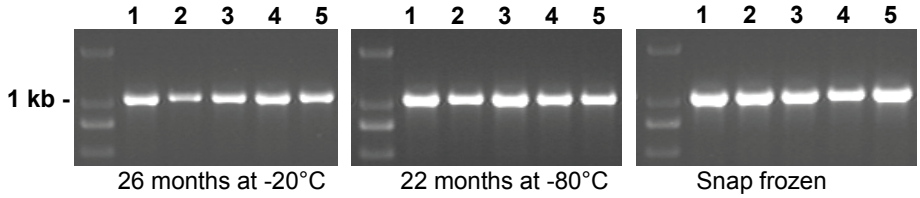

Figure 5. End-point RT-PCR of a 1 kb gene fragment. A 1065 bp sequence of the rat HPRT mRNA was analyzed by one-step, end-point RT-PCR with RNA from **1** rat liver, **2** kidney, **3** spleen, **4** intestine, and **5** lung. The RNA was isolated from PFPE tissue stored prior to processing for 26 months at –20°C or for 22 months at –80°C as well as from tissue snap frozen in LN2. Amplification was performed with 10 ng RNA using the QIAGEN® OneStep RT-PCR Kit.

In addition to end-point RT-PCR, RNA isolated from PFPE tissues was tested for performance in real-time RT-PCR using a primer/probe assay for amplification of a 294 bp fragment of β-actin mRNA. The C_T values obtained with RNA from PFPE tissues differed from the C_T values obtained with reference RNA by approximately –1 to +1.5 C_T (Figure 6). No significant increase in C_T values relative to tissue storage time or temperature was observed.

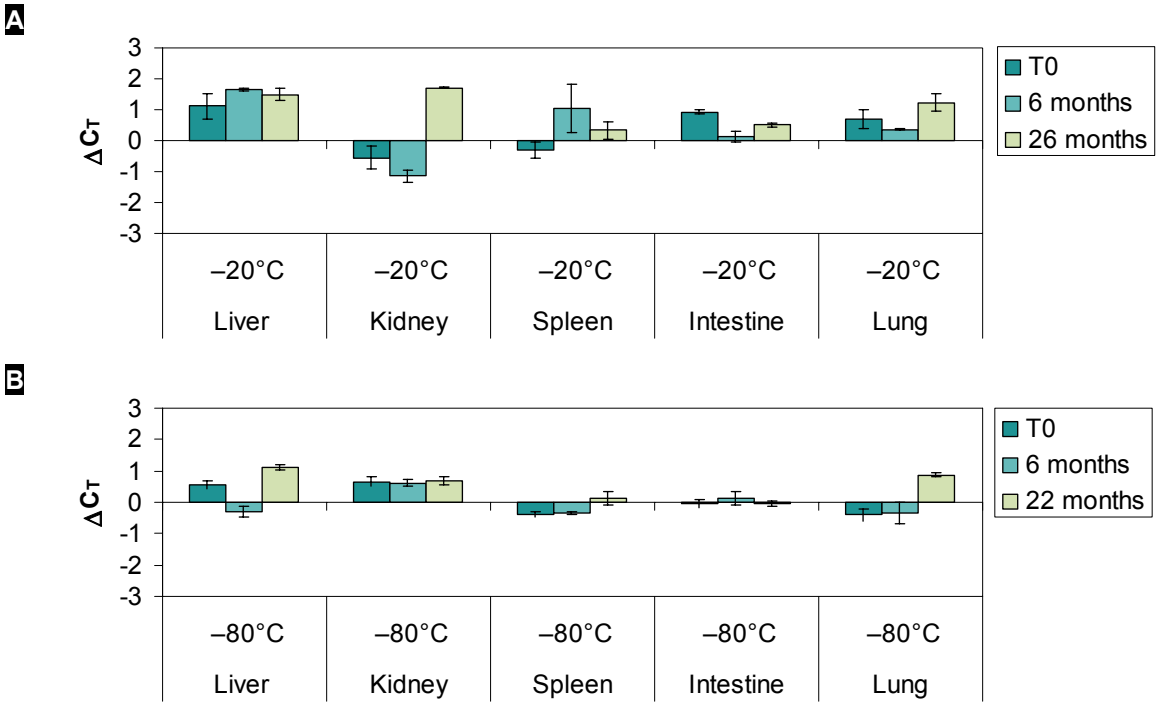

Figure 6. β-Actin real-time RT-PCR. Gene expression of β-actin was analyzed in a quantitative real-time RT-PCR assay by amplification of a 294 bp fragment. RNA was extracted in triplicate from PFPE tissue stored **A** for up to 26 months at –20°C or **B** for 22 months at –80°C and amplified in duplicate using the QuantiTect® Probe RT-PCR Kit (QIAGEN) with 10 ng RNA. Assay results were compared with results using reference RNA from the corresponding tissues snap-frozen in liquid nitrogen. $\Delta C_T = C_T$(RNA from PFPE) – C_T(RNA from T0). **T0**: Time-zero reference PFPE samples directly processed without storage.

Conclusion

Tissue samples fixed in the PAXgene Tissue Container can be stored in the PAXgene Tissue Stabilizer for up to 26 months at –20°C or for up to 22 months at –80°C (storage studies are ongoing). After processing and paraffin embedding, tissue morphology can be analyzed by H&E staining, and intact RNA can be isolated from the PFPE tissue that performs well in end-point and real-time RT-PCR.

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.

For up-to-date licensing information and product-specific disclaimers, see the respective PreAnalytiX® or QIAGEN kit handbook or user manual. PreAnalytiX and QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

www.PreAnalytiX.com

PreAnalytiX GmbH, 8634 Hombrechtikon, CH.