



Technical Note PAXgene[®] Tissue System

RNA stability in tissue samples, fixed and stabilized with the PAXgene Tissue System, for up to 7 days at 22°C or 2 months at 4°C

Introduction

The PAXgene Tissue Container is comprised of 2 reagents, a fixation and a stabilization reagent, in different chambers of a single container. The fixation reagent, PAXgene Tissue Fix, preserves morphology and the integrity of biomolecules. A minimum fixation time of 2 hours is recommended for specimens with a maximum size of 4 x 10 x 10 mm. Prolonged incubation in the fixation reagent can lead to degradation of biomolecules, especially RNA. Therefore, it is recommended to stop the fixation process by transferring the tissue specimen from the fixation reagent into the stabilization reagent, PAXgene Tissue Stabilizer, within 24 hours after fixation. Longer fixation times may be possible but must be validated by the user.

In this study, we investigated the quality of RNA from rat tissue fixed with PAXgene Tissue Fix for either 3 or 24 hours and then stored in PAXgene Tissue Stabilizer at 22°C for up to 7 days or at 4°C for up to 2 months.

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. Within 5 minutes after resection, tissue samples were fixed in PAXgene Tissue Fix for 3 or 24 hours at room temperature. Fixation was stopped by transferring the samples into PAXgene Tissue Stabilizer. Samples were stored in PAXgene Tissue Stabilizer for up to 7 days at 22°C or up to 60 days at 4°C (Figure 1). Tissue samples were

removed from the PAXgene Tissue Stabilizer at different time points, and RNA was purified in triplicate from 20 mg tissue samples using the PAXgene Tissue RNA Kit according to the protocol “Purification of Total RNA from PAXgene Treated Tissue Samples” in the *PAXgene Tissue RNA Kit Handbook* (April 2009). Tissue disruption and homogenization were carried out using the QIAGEN[®] TissueLyser II.

RNA yield and purity were analyzed by measuring the absorbance at 260, 280, and 320 nm on a NanoDrop[®] spectrophotometer. RNA integrity was analyzed on an Agilent[®] Bioanalyzer with the Agilent RNA 6000 Nano assay. Performance in real-time RT-PCR was analyzed using a TaqMan[®] primer/probe assay for β -actin.

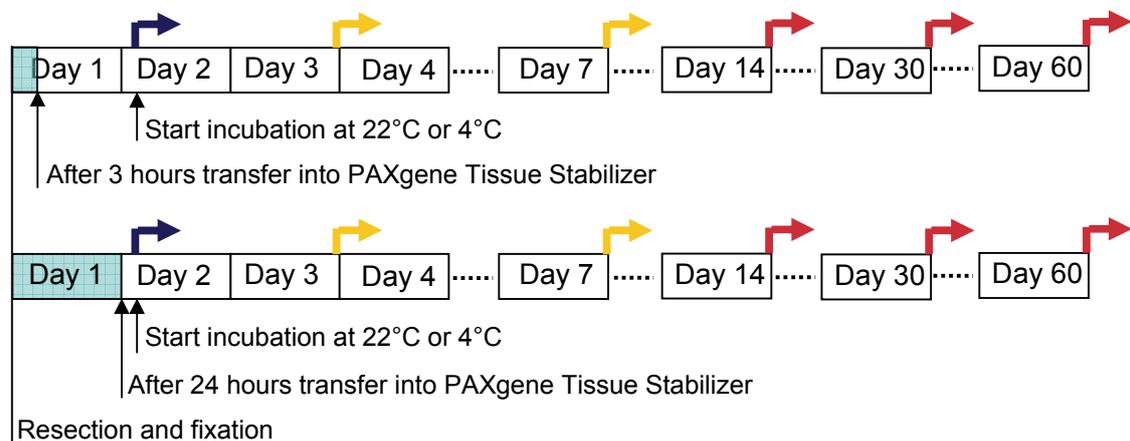


Figure 1. Study workflow. Rat tissue was fixed in PAXgene Tissue Fix for 3 or 24 hours at room temperature. Fixation was stopped by transferring tissue samples into PAXgene Tissue Stabilizer. One day after resection, samples were incubated in PAXgene Tissue Stabilizer at either 22°C or 4°C. RNA was directly purified from stabilized tissue at the beginning of the incubation phase, after day 1 (blue arrow: RNA used as reference in RT-PCR, Figure 4), after days 3 and 7 of 22°C storage (yellow arrow), and after days 14, 30, and 60 of 4°C storage (red arrow).

Results

RNA yield was generally high but varied according to tissue type, from 10 μ g for lung tissue to 40 μ g for liver tissue. Fixation time (3 or 24 hours) as well as storage periods and temperature during storage had only a minor effect on yield (Figure 2).

A_{260}/A_{280} absorbance ratios were between 2.0 and 2.1 for all RNA samples, regardless of fixation and storage periods, indicating that purity of the RNA was high.

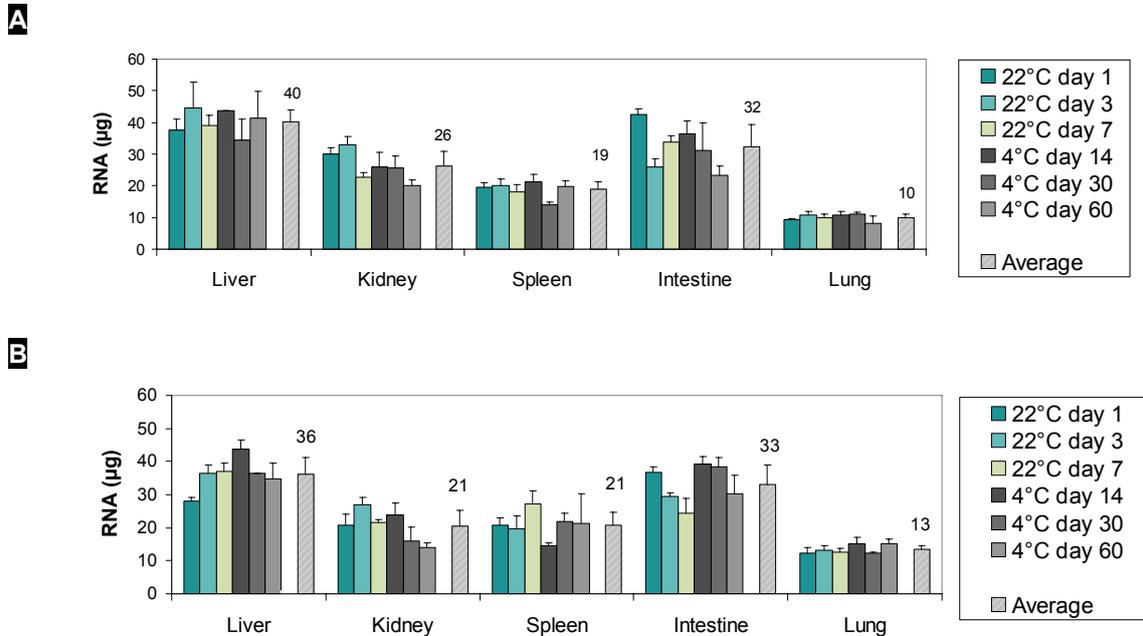


Figure 2. RNA yield from 20 mg of PAXgene Tissue fixed and stabilized tissue. RNA yield was analyzed by spectrophotometry using a NanoDrop spectrophotometer. RNA was isolated from triplicate tissue samples fixed in PAXgene Tissue Fix for **A** 3 hours or **B** 24 hours and stored in PAXgene Tissue Stabilizer at the temperatures indicated for the indicated time periods.

RNA integrity numbers (RIN), determined by analysis with the Agilent Bioanalyzer, were consistent within the fixation and stabilization periods tested but varied between tissue types. Except for kidney, for which RIN values varied from 7.3 to 5.3, RIN values were consistently above 7. The highest RIN values, from 8.7 to 9.9, were for RNA from intestine (Figure 3).

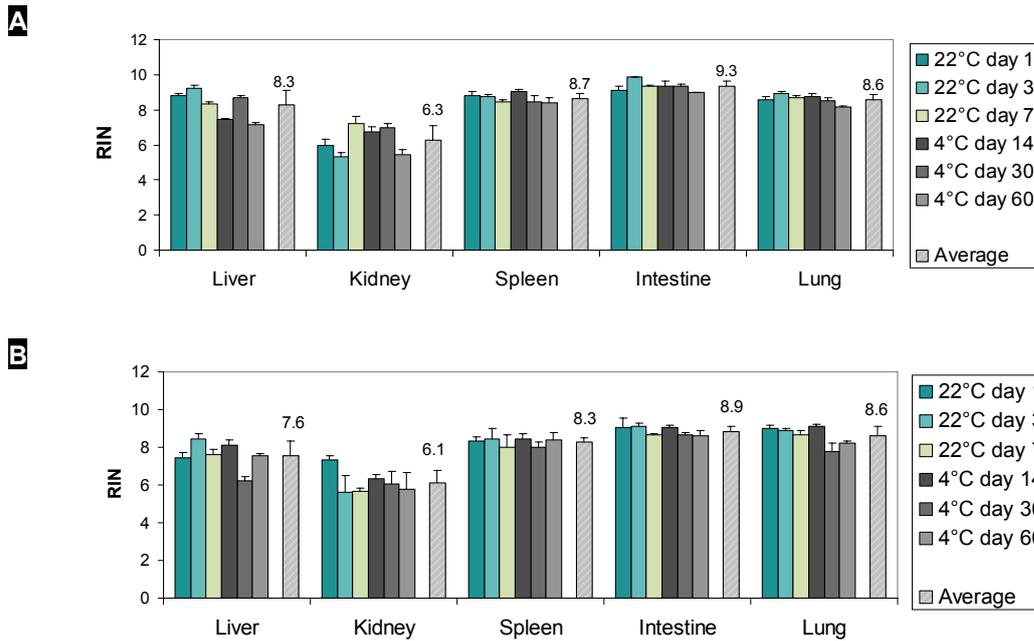


Figure 3. RNA integrity. RNA integrity was analyzed with an Agilent Bioanalyzer to give the RNA integrity number (RIN). RNA was isolated from triplicate tissue samples fixed in PAXgene Tissue Fix for **A** 3 hours or **B** 24 hours and stored in PAXgene Tissue Stabilizer at the temperatures indicated for the indicated time periods.

To test performance in real-time RT-PCR, a 294 bp fragment of β -actin was amplified using 10 ng of total RNA in a TaqMan primer/probe assay. A delta C_T value (ΔC_T) was calculated by comparing C_T values obtained with RNA from stored samples and the C_T values obtained with RNA isolated immediately from samples one day after resection (see Figure 1).

ΔC_T values ranged from 0.9 to -1.4 (Figure 4), indicating that there was no significant increase in C_T values corresponding to various tissue fixation and storage times or temperatures during storage.

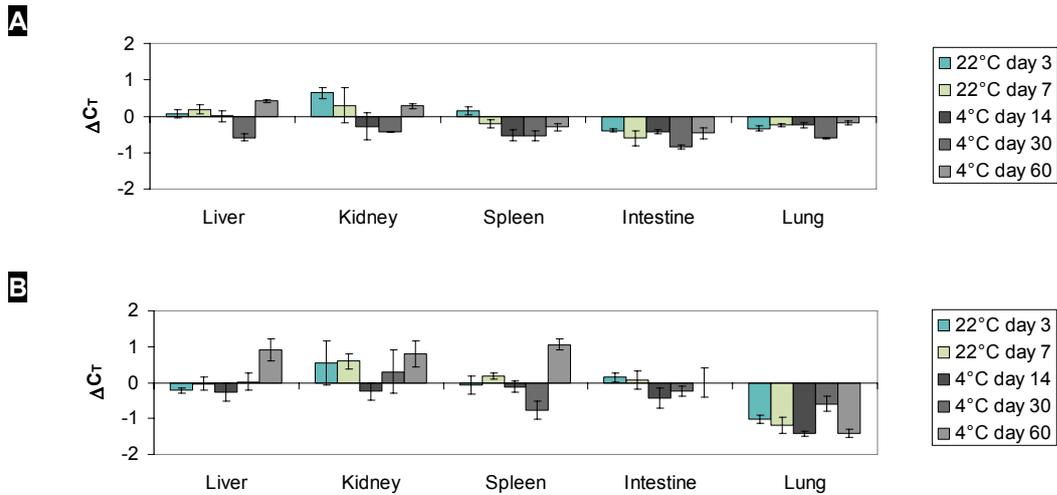


Figure 4. β -Actin real-time RT-PCR. Gene expression analysis of a 294 bp fragment of β -actin RNA by quantitative real-time RT-PCR. RNA extraction was performed from triplicate tissue samples fixed with PAXgene Tissue Fix for **A** 3 hours or **B** 24 hours and stored in PAXgene Tissue Stabilizer at the temperatures indicated for the indicated time periods. Purified RNA (10 ng) was amplified in duplicate using the QIAGEN QuantiTect[®] Probe RT-PCR Kit. C_T values obtained with RNA from stored samples were compared with C_T values obtained with RNA isolated immediately from samples one day after resection (see also Figure 1). $\Delta C_T = C_T^{\text{RNA from tissue stored at 22}^\circ\text{C or 4}^\circ\text{C}} - C_T^{\text{RNA from tissue day 1 after resection}}$.

Conclusion

High-quality, high-molecular-weight RNA, which performs well in real time RT-PCR, can be isolated from rat liver, kidney, spleen, intestine, and lung tissue samples fixed and stored with the PAXgene Tissue System for up to 7 days at 22°C or up to 2 months at 4°C.

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