# Technical Note
# PAXgene® Tissue System

## Vacuum sealing of fixed and stabilized tissue with a FoodSaver® Vacuum Sealer for dry and safe transport

### Study design

Rat (*Rattus norvegicus*) tissue specimens from liver, kidney, spleen, intestine, lung, and cerebrum were excised and cut into pieces of approximately 4 x 15 x 15 mm, and placed into tissue cassettes with square mesh lids (to prevent them from deforming under vacuum). Fixation was performed for 4 hours with the PAXgene Tissue FIX Container (50 ml) (cat. no. 765312) and was stopped by exchanging the fixative with PAXgene Tissue STABILIZER (cat. no. 765512). After incubation for 24 hours, cassettes with tissue samples were removed from the PAXgene Tissue STABILIZER, placed into FoodSaver bags, and vacuum sealed with a FoodSaver Vacuum Sealer (e.g., FoodSaver V3840, in the US, or FoodSaver V2860, in the EU, from Sunbeam Products, Inc.). To simulate transportation, FoodSaver bags were stored for 7 days at 22°C. For comparison, additional samples were stored in PAXgene Tissue STABILIZER for 7 days at 22°C without vacuum.

After storage, cassettes with fixed and stabilized tissues were removed from the bags or containers and directly placed into a tissue processor (TP1020, Leica Biosystems). Tissue was processed as follows: incubation at 80%, 90%, 99% ethanol (2x), followed by isopropanol (2x), xylene (2x), xylene mixed 1:1 with low melting paraffin, and infiltration and embedding in low melting paraffin (2x) for no longer than 1 hour at each position, resulting in PAXgene Tissue fixed, paraffin-embedded (PFPE) tissues (Figure 1).

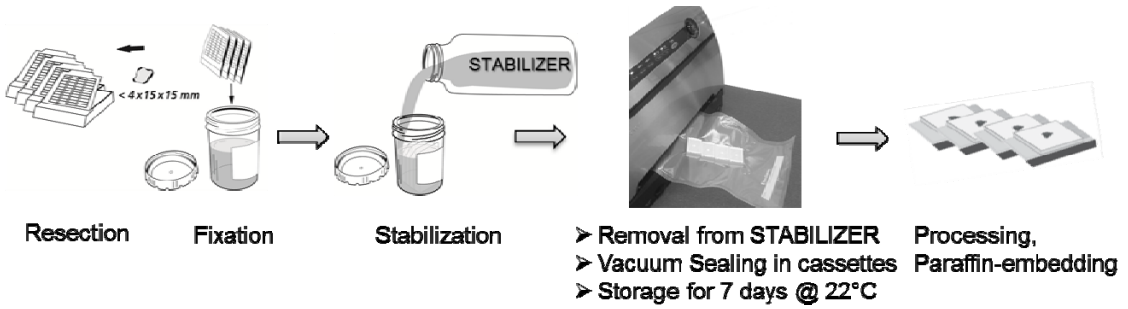



**Figure 1. Vacuum sealing in tissue cassette.** Rat tissue samples were fixed in tissue cassettes with the PAXgene Tissue FIX Container (50 ml). The fixative was replaced by PAXgene Tissue STABILIZER and tissue samples were vacuum sealed within the cassettes with a FoodSaver Vacuum sealer. After incubation in FoodSaver bags for 7 days at 22°C, the samples were processed and paraffin embedded.

For analysis of morphology, 4 µm sections from PFPE tissues were stained with hematoxylin and eosin (H&E). RNA was purified in triplicate from 3 sections of PFPE tissues (10 µm thick) using the PAXgene Tissue RNA Kit. 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. Performance in real-time RT-PCR was assessed with a proprietary TaqMan® Primer/Probe assay for the β-actin gene.

## Results

Incubation for 7 days at 22°C under vacuum in FoodSaver bags had no adverse effects on PFPE tissue morphology. Morphology of H&E stained sections was intact and without artifacts (Figure 2).

RNA total yield from three 10 µm sections of PFPE tissue varied according to tissue type: the lowest yield, 1 µg, was from lung and the highest, 8 µg, was from liver. After normalization (ng RNA per square millimeter of 10 µm section PFPE), only minor variation in yield was observed between the storage conditions (Figure 3). RNA integrity was consistently high for all samples: RNA integrity numbers (RIN) above 7 were obtained from all samples except kidney, which exhibited RINs in the range of 6.5–7.0 (Figure 4). Average RIN values from tissue stored under two conditions did not vary significantly (T-test of two samples assuming equal variances resulted in $p [T \leq t] = 0.749$).

Differences in $C_T$ values of 10 ng RNA from samples stored under the two conditions were consistently < 1, which is below the level of assay precision, and thus insignificant (Figure 5).

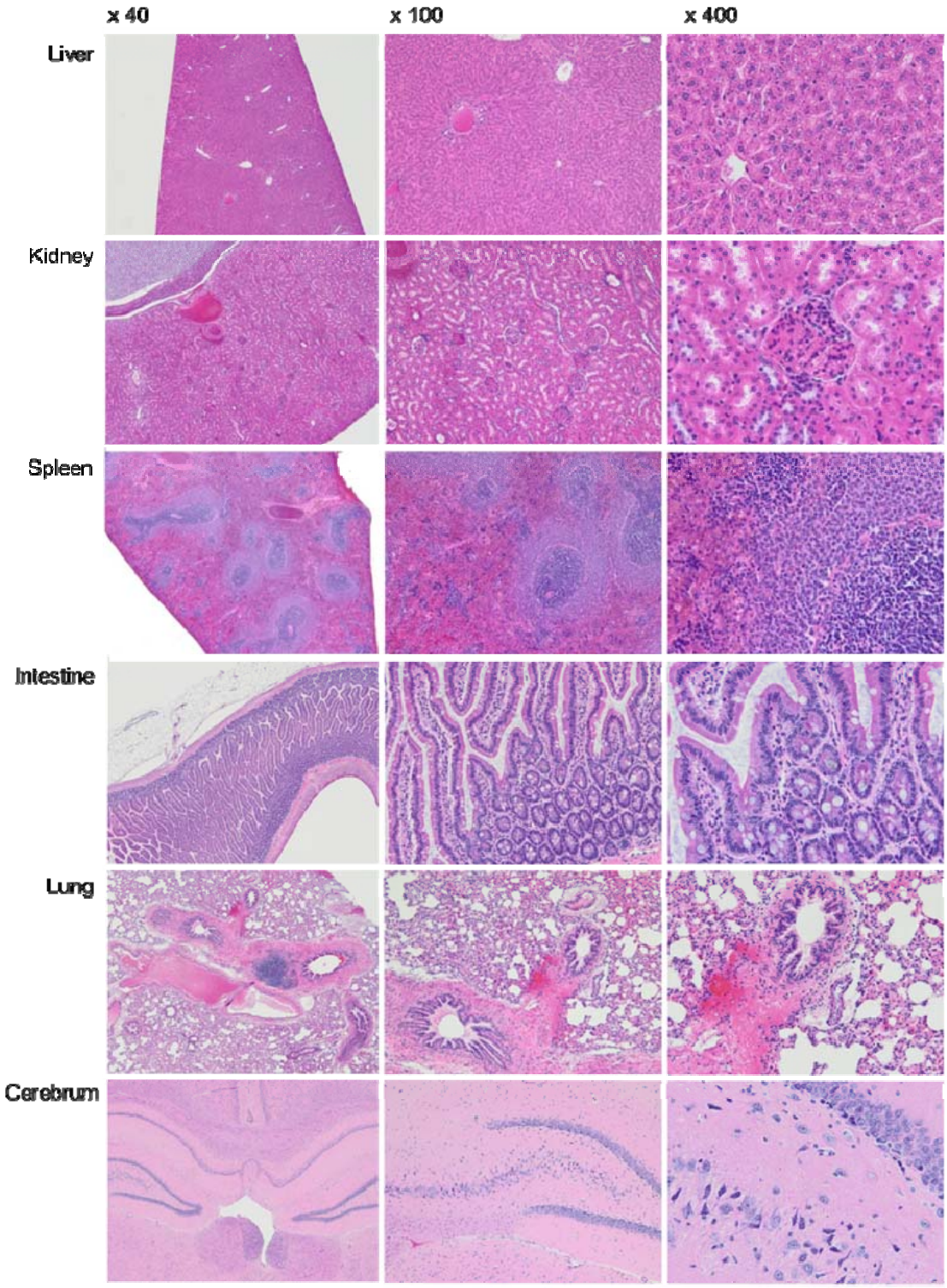
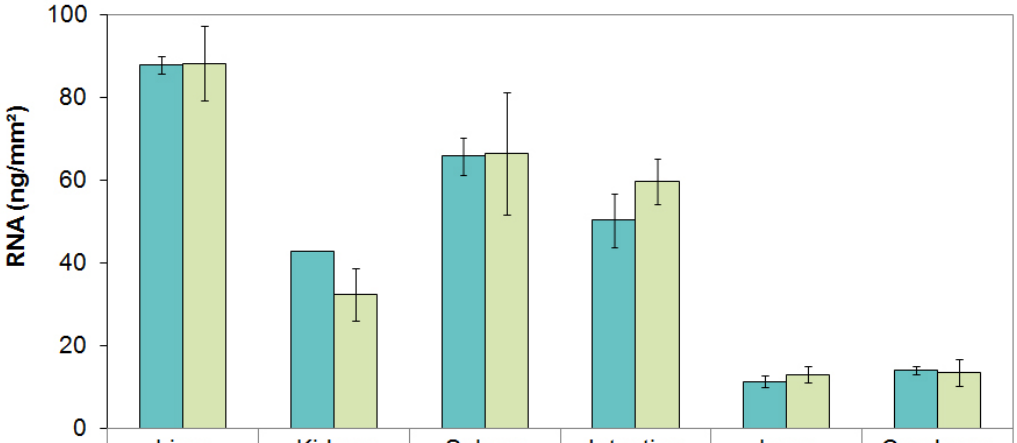
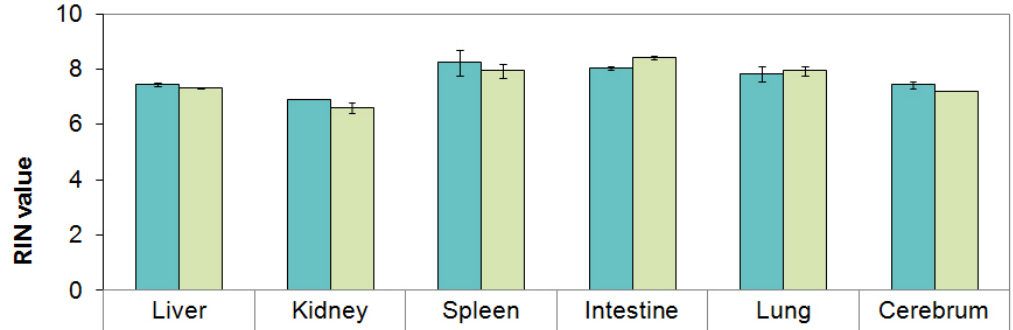



**Figure 2. Preservation of morphology.** Hematoxylin and eosin staining of 4 µm sections of rat PFPE liver, kidney, spleen, intestine, lung, and cerebrum tissue. Tissue was fixed four 4 hours, stabilized for 24 hours, vacuum sealed within histocassettes in FoodSaver bags, and stored for 7 days at 22°C prior to processing. Original magnification was at x40, x100 and x400.



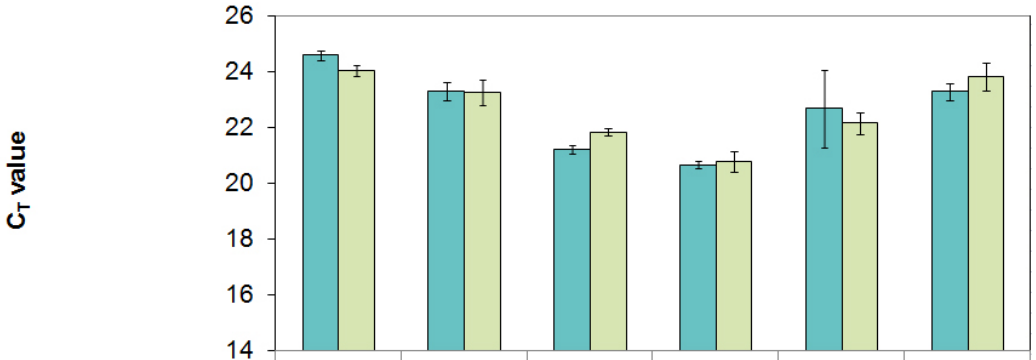

| | Liver | Kidney | Spleen | Intestine | Lung | Cerebrum |
|---|---|---|---|---|---|---|
| FoodSaver bag | 88 | 43 | 66 | 50 | 11 | 14 |
| PAXgene Tissue STABILIZER | 88 | 32 | 66 | 60 | 13 | 13 |

**Figure 3. RNA yield (ng/mm²) from PFPE tissue sections.** Yield analyzed by spectrophotometry using a NanoDrop® instrument.




| | Liver | Kidney | Spleen | Intestine | Lung | Cerebrum |
|---|---|---|---|---|---|---|
| FoodSaver bag | 7.4 | 6.9 | 8.2 | 8.0 | 7.8 | 7.4 |
| PAXgene Tissue STABILIZER | 7.3 | 6.6 | 7.9 | 8.4 | 7.9 | 7.2 |

**Figure 4. RNA integrity (RIN) values.** RNA integrity was analyzed with an Agilent Bioanalyzer. Mean value and standard deviations were calculated from triplicate samples according to tissue type and treatment.




| | Liver | Kidney | Spleen | Intestine | Lung | Cerebrum |
|---|---|---|---|---|---|---|
| FoodSaver bag | 24.6 | 23.3 | 21.2 | 20.7 | 22.7 | 23.3 |
| PAXgene Tissue STABILIZER | 24.1 | 23.3 | 21.9 | 20.8 | 22.2 | 23.8 |

**Figure 5. Real-time RT-PCR of the β-actin gene.** Gene expression of β-actin was analyzed in a quantitative real-time RT-PCR assay by amplification of a 294 bp fragment using the QuantiTect® Probe RT-PCR Kit (QIAGEN). Error bars represent 6 assays: tissue samples were purified in triplicate, and 10 ng from each RNA extraction was tested in duplicate.

## Conclusion

- Samples of various tissue types (liver, kidney, spleen, intestine, lung, and cerebrum) from rat that have been fixed and stabilized with the PAXgene Tissue System can be vacuum sealed in FoodSaver bags for transportation *within a tissue cassette* using a FoodSaver vacuum sealer.
- No significant differences were observed in tissue morphology, RNA yields, and performance in RT-PCR from PFPE tissue prepared from samples that were stored 7 days at 22°C either under vacuum in a FoodSaver bag or in PAXgene Tissue STABILIZER.

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