

Storage of Tissue in PAXgene® Tissue STABILIZER: RNA and Morphology Preservation after 5 Years at -20 and 3 years at -80°C

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Methods

- Cryopreservation
 - Snap freezing in liquid nitrogen
 - Storage at -80°C
 - RNA extraction with RNeasy Mini Kit
 - 10 mg tissue, homogenization with Tissue Lyzer
- PAXgene Tissue
 - 4 h fixation, 24 h stabilization at room temp.
 - Storage in 2 ml screw cap tubes filled with STABILIZER
 - RNA extraction with PAXgene Tissue RNA Kit
 - PF: 10 mg tissue, homogenization with Tissue Lyzer
 - PFPE: 4 x 10 µm sections
- RNA analysis
 - Yield: Nanodrop Spectrophotometer
 - Integrity: Agilent Bioanalyzer
 - RT-qPCR: primer/probe one step RT-PCR assay (294 nt) of rat β-actin gene;
 - ΔC_T calculation: C_T (PFPE) - C_T (Cryo)

Conclusion

- PAXgene Tissue fixed/ stabilized samples can be stored within the STABILIZER at -20 to -80°C
- Within the STABILIZER morphology and RNA is preserved for up to 57 months at -20°C and up to 36 months at -80°C (long-term storage study ongoing)

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Results

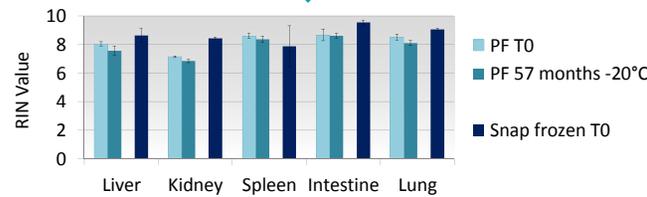
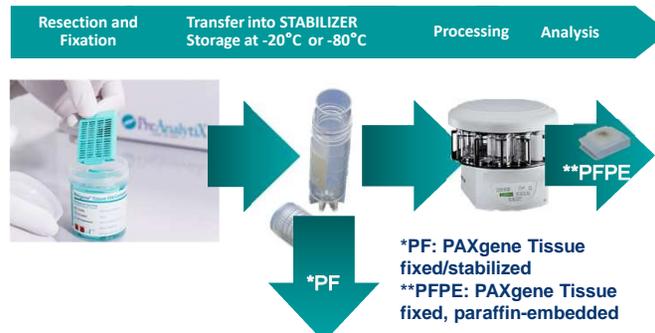


Fig. 1: RNA integrity from rat tissue PF samples, before (T0) and after storage in PAXgene Tissue STABILIZER for 57 months at -20°C, and from snap frozen tissue.

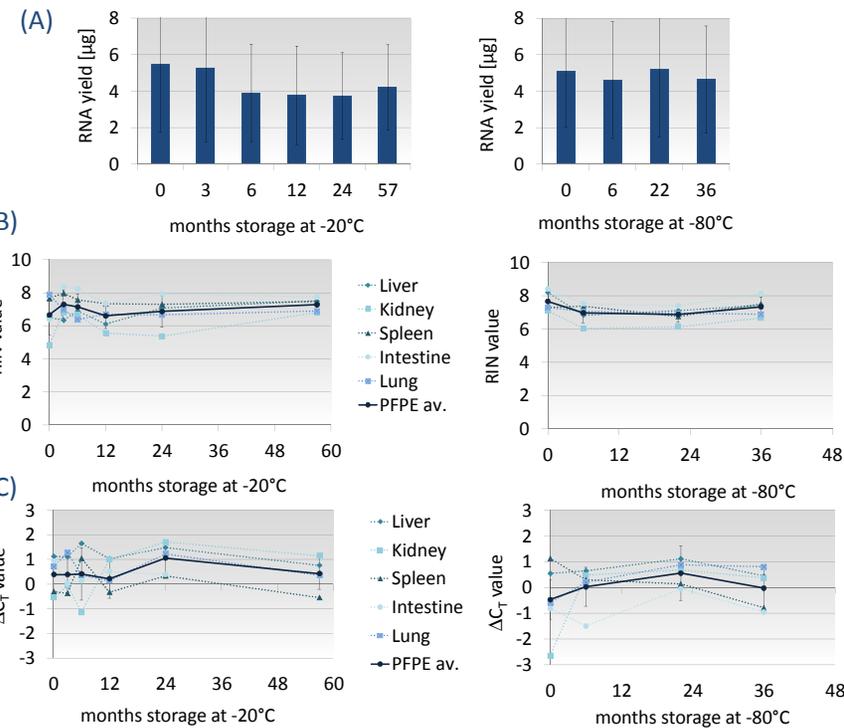
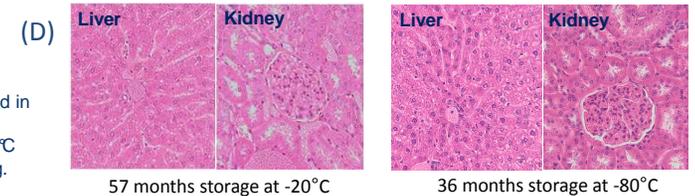


Fig. 2: RNA yield (A), integrity (B), performance in RT-qPCR (C), and H&E morphology (D) from sections of rat PFPE samples, fixed with PAXgene Tissue, stored in PAXgene Tissue STABILIZER for up to 57 months at -20°C, or up to 36 months at -80°C prior to processing and paraffin embedding.



Morphology and RNA is preserved in PAXgene Tissue fixed and stabilized samples stored within the STABILIZER at -20 to -80°C

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Methods

- Cryopreservation
 - Snap freezing in liquid nitrogen
 - Storage at -80°C, with or without additional incubation at 22°C
 - RNA extraction with RNeasy Mini Kit
 - 10 mg tissue, homogenization with Tissue Lyzer
- PAXgene Tissue
 - 4 h fixation, 24 h stabilization at room temp.
 - Storage in 2 ml screw cap tubes filled with STABILIZER at -80°C, with or without repeated thawing and freezing cycles
 - RNA extraction with PAXgene Tissue RNA Kit
 - 10 mg tissue, homogenization with Tissue Lyzer
- RNA analysis
 - Yield: Nanodrop Spectrophotometer
 - Integrity: Agilent Bioanalyzer
 - RT-qPCR: primer/probe one step RT-PCR assay (294 nt) of rat β -actin gene

Results

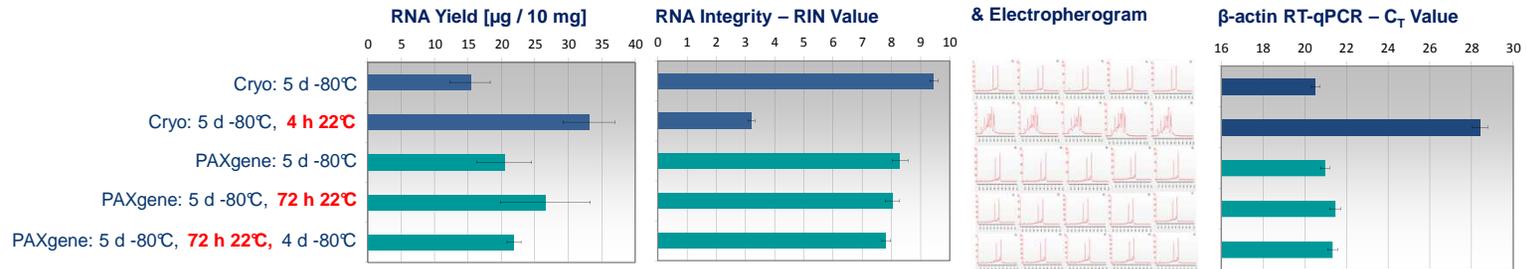


Fig. 1: Rat liver tissue samples (4 x 4 x 10 mm) snap frozen in liquid nitrogen (Cryo) or fixed with PAXgene Tissue and stored in PAXgene Tissue STABILIZER at -80°C. RNA extracted from frozen tissue or from frozen and thawed tissue as indicated.

Conclusion

- RNA and morphology of tissue samples fixed and stabilized with the PAXgene Tissue System and stored within the STABILIZER at -80°C are stable even in case of repeated freezing and thawing cycles.
- Incubation of PAXgene Tissue fixed rat liver stored within the STABILIZER at -80°C for three days at room temperature prior to RNA purification has no significant impact on RNA yield, integrity and performance in RT-qPCR.

PAXgene (PFPE):
 8 d -80°C
 72 h 22°C
 22 d -80°C
 72 h 22°C
 processing
 H&E staining

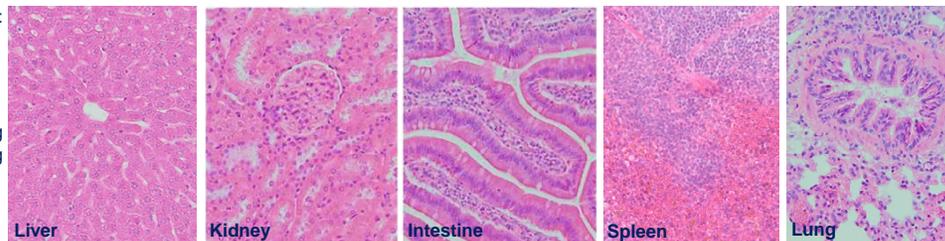


Fig. 2: Rat liver tissue samples fixed with PAXgene Tissue and stored in PAXgene Tissue STABILIZER at -80°C. RNA extracted directly from frozen PF tissue (T0), or from frozen PF tissue thawed and incubated at 22°C for 24 h (T1) or 72 h (T2) prior RNA extraction; 20 replicates each.

Fig. 3: Rat tissue samples fixed with PAXgene Tissue, stored in PAXgene Tissue STABILIZER at -80°C, incubated for 72 h at 22°C, frozen again and incubated for additional 72 h at 22°C prior to processing, paraffin embedding and staining with Hematoxylin & Eosin.

PAXgene Tissue fixed and stabilized (PF) samples stored within the STABILIZER withstand repeated thawing and freezing cycles

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