Technical Note
PAXgene™ Blood RNA System

*In Situ* Stability of RNA in Blood Samples Stored at -20 and -70°C in PAXgene Blood RNA Tubes

**Study Design**

For each study, blood was drawn into PAXgene tubes from a minimum of ten consenting adult donors with white blood cell (WBC) counts in the normal range of 4.8 – 11.0 x 10⁶ WBC/ml blood. Replicate specimens were stored *in situ* at either -20°C or -70°C and processed in duplicate at the indicated time points* in accordance with the PAXgene Blood RNA Kit Handbook.

Purified RNA was analyzed for integrity† using the Agilent Bioanalyzer and tested in qRT-PCR assays for FOS and IL1B.

**Results**

**Stability of RNA in Blood Stored *in situ* at -20°C**

Figures 1A and 1B depict the change in relative FOS and IL1B transcript number respectively for RNA in blood stored *in situ* in PAXgene Blood RNA Tubes at -20°C.

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* An additional study is continuing for 10 years.
† RNA integrity results provided for supporting data only; no claims for RNA integrity are made for the PAXgene Blood RNA System.
Figure 1A: Relative transcript levels of FOS in RNA purified from blood stored in situ at -20°C in PAXgene Blood RNA Tubes. Blood, collected from 10 donors, each with two RNA sample preparation replicates, was analyzed. The means and standard deviations of all storage test time points are plotted as orange lines with black vertical bars. Assay precision (±3 x total precision of the assay with consideration of single data (VVS = |3 x σT| = 2.34 C_T)) is shown as dashed red lines.

Figure 1B: Relative transcript levels of IL1B in RNA purified from blood stored in situ at -20°C in PAXgene Blood RNA Tubes. Blood, collected from 10 donors, each with two RNA sample preparation replicates, was analyzed. The means and standard deviations of all storage test time points are plotted as orange lines with black vertical bars. Assay precision (±3 x total precision of the assay with consideration of single data (VVS = |3 x σT| = 1.93 C_T)) is shown as dashed red lines.
There were no significant changes in the relative transcript level of FOS or IL1B due to *in situ* storage of whole blood in PAXgene Blood RNA Tubes at -20°C for up to 50 months. All variations in the ΔΔCT values stayed within the range of ±3 x the total precision of the assay with consideration of single data (FOS: VVS = | 3 x σ | = 2.34 CT; IL1B: VVS = | 3 x σ | = 1.93 CT).

Figure 2 depicts RNA integrity numbers (RINs) for RNA purified from blood stored in PAXgene Blood RNA Tubes at -20°C for 50 months. Using the PAXgene Blood RNA Kit, blood samples from 10 donors were processed in duplicate after the indicated blood storage times.

No significant loss of RNA integrity was detected in whole blood samples stored for 50 months at -20°C in PAXgene Blood RNA Tubes.

**Stability of RNA in Blood Stored in situ at -70°C**

Figures 3A and 3B depict the change in relative FOS and IL1B transcript number respectively for RNA in blood stored *in situ* in PAXgene Blood RNA Tubes at -70°C.
**Figure 3A:** Relative transcript levels of FOS in RNA purified from blood stored *in situ* at -70°C in PAXgene Blood RNA Tubes. Blood, collected from 10 donors, each with two RNA sample preparation replicates, was analyzed. The means and standard deviations of all storage test time points are plotted as orange lines with black vertical bars. Assay precision ($\pm 3 \times$ total precision of the assay with consideration of single data ($VVS = | 3 \times \sigma_{T} | = 2.34 C_{T}$)) is shown as dashed red lines.

**Figure 3B:** Relative transcript levels of IL1B in RNA purified from blood stored *in situ* at -70°C in PAXgene Blood RNA Tubes. Blood, collected from 10 donors, each with two RNA sample preparation replicates, was analyzed. The means and standard deviations of all storage test time points are plotted as orange lines with black vertical bars. Assay precision ($\pm 3 \times$ total precision of the assay with consideration of single data ($VVS = | 3 \times \sigma_{T} | = 1.93 C_{T}$)) is shown as dashed red lines.
There were no significant changes in the relative transcript level of FOS or IL1B due to *in situ* storage of whole blood in PAXgene Blood RNA Tubes at -70°C for up to 50 months. All variations in the $\Delta\Delta C_T$ values stayed within the range of $\pm 3 \times$ the total precision of the assay with consideration of single data (FOS: VVS = $| 3 \times \sigma | = 2.34 \ C_T$; IL1B: VVS = $| 3 \times \sigma | = 1.93 \ C_T$).

Figure 4 depicts RNA integrity numbers (RINs) for RNA purified from blood stored in PAXgene Blood RNA Tubes at -70°C for 50 months. Using the PAXgene Blood RNA Kit, blood samples from 10 donors were processed in duplicate after the indicated blood storage times.

![Figure 4: Integrity of RNA purified from whole blood stored in PAXgene Blood RNA Tubes at -70°C. Means of RINs of duplicates of all samples from all donors are presented as columns. The ends of upper and lower bars indicate the individual RINs of duplicate samples or, in the case of means, the standard deviations of RINs of all samples from all donors. Blood samples of donor 5 were excluded from study due to invalid WBC counts which were outside of the stated acceptance criteria. Data sets of donors 6 and 8 (not shown) were also excluded due to an increase in RIN values which were outside of the normal variability of this measurement. Based on a Dixon test (level of significance alpha = 1%), these higher than expected RIN values were identified as outliers and were most likely the results of user error. The sets of data containing outliers were therefore excluded from calculation and presentation.

No significant loss of RNA integrity was detected in whole blood samples stored for 50 months at -70°C in PAXgene Blood RNA Tubes.
Conclusion

Blood can be stored *in situ* in PAXgene Blood RNA Tubes for at least 50 months at either -20°C or -70°C without loss of function in qRT-PCR analysis.

Furthermore, supplementary data indicated that, for duplicate measurements of multiple donors, mean values for RINs were between 7 and 8 at all time points between zero and 50 months.

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PreAnalytiX GmbH, 8634 Hombrechtikon, CH.

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