Stability of Extracted RNA at Various Storage Temperatures and through Multiple Freeze-Thaw Cycles

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ABSTRACT

Background: Degradation during storage of extracted RNA may make the sample unusable for downstream assays such as reverse transcription, in vitro translation, differential display, and expression-array and expression-chip analysis. It is thought that RNA degradation during storage results primarily from base hydrolysis of RNA which occurs under either low pH conditions or conditions where divalent cations catalyze hydrolysis. We investigated the effect of different storage temperatures and freeze-thaw regimens on RNA stability during storage.

Methods: RNA from a tissue sample was extracted using the Qiagen RNeasy Kit according to the manufacturer's protocol. The concentration was adjusted to approximately 300 µg/mL using ultra-pure water. The RNA was aliquoted into single use aliquots and stored at room temperature, 4ºC, or -20ºC. These samples were tested at an initial time point and after 1, 2, 3, 7, 14, or 28 days of storage. Test parameters included concentration, ribosomal RNA ratio (28S:18S), and RIN number, and testing was performed in duplicate. Similarly, a subset of samples were placed at either -80ºC or -20ºC and subjected to up to ten freeze-thaw cycles.

Results: RNA stored at room temperature was stable through 7 days, but showed declines in all three test parameters at 14 and 28 days. No declines in RNA concentration, rRNA ratio, or RIN number were observed for RNA stored at either 4ºC, or -20ºC. Regardless of whether the RNA was cycled between -80ºC and room temperature or -20ºC and room temperature, up to 10 freeze-thaw cycles did not result in any concentration or integrity decline.

Conclusion: This study suggests that the purity of the RNA resulting from the extraction process, and the pH and composition of the RNA diluent, play the critical role in preventing degradation upon storage. When pure RNA is stored in an appropriate buffer, it is resistant to degradation under sub-optimal conditions of the type likely to be encountered during accidental mishandling or shipping delays.

MATERIALS AND METHODS

RNA Extraction: A good quality tissue was chosen for this study. The total RNA from the frozen tissue was extracted using the Qiagen RNeasy Kit with column DNase treatment. The RNA was dissolved in RNase-free water and the concentration and yields were quantitated by OD reading at 260 nm using the SpectraMax Plus Spectrophotometer (Molecular Devices). The RNA concentration was adjusted to 324 µg/mL.

Analysis of Extracted RNA for Quality Control: The Agilent Bioanalyzer was used to determine the quality and quantity of the extracted RNA. The RIN (RNA Integrity Number) and ribosomal RNA ratio (28S:18S) indicate whether the extracted RNA is of high quality. At the start of the study, the RNA had a RIN of 8.1, indicating excellent integrity. Each sample was retested after completion of storage in the stress condition.

Storage and Stability Testing: Normalized RNA was aliquoted into multiple tubes at 15 µL per tube. The tubes were then moved to the respective test conditions for the study (Table 1).

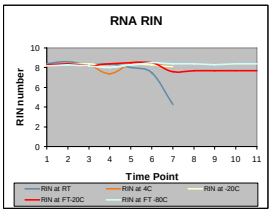
- The samples for real time stability testing were stored at room temperature, 4°C, or -20°C for up to 28 days. At each storage time point (see table 1), 2 aliquots were removed and transferred to -80°C for future testing as a batch. SeraCare has previously demonstrated that storage at -80°C preserves RNA indefinitely. (Data not shown.)
- Samples for the freeze-thaw study were placed at -20°C or -80°C. The tubes were labeled as 1FT, 2FT … 10FT. Tubes were removed from -20°C and -80°C storage, thawed at RT for about 10 minutes, and replaced at -20°C or -80°C to re-freeze at least overnight. This was repeated until all freeze-thaws for that sample had been completed. Two tubes were processed for each condition.

Table 1: Parameters to Analyze the Stability of Total RNA

	Stress Conditions	Test Intervals
Real time stability of RNA incubated at various temperatures	Room Temperature	0, 1, 2, 3, 7, 14, & 28 Days
	4°C	
	-20°C	
Stability of RNA through multiple freeze-thaw cycles (freezing at two different temperatures)	-20°C	0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 Freeze Thaw Cycles
	-80°C	

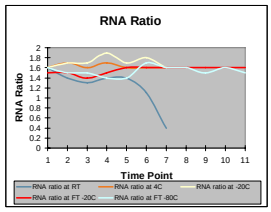
RESULTS

Figure 1: Stability of RNA Integrity (RIN) over Time



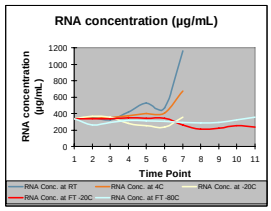
Tissue RNA integrity is not affected by the storage conditions in 14 days, and not affected by freeze-thaws up to 10 cycles. It was degraded by 28 days at room temperature.

Figure 2: Stability of rRNA Ratio over Time



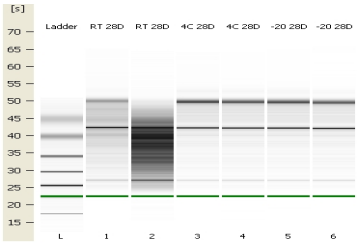
The ribosomal RNA ratio declined with storage at room temp. The ratio was not affected by storage 4°C, -20°C, or by up to 10 freeze-thaw cycles.

Figure 3: Stability of RNA Concentration over Time



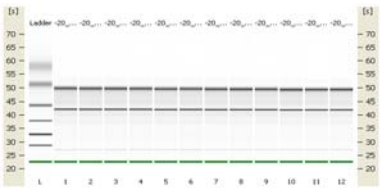
The RNA concentration increased over time at both room temperature and 4°C, showing that evaporation occurred at room temperature from day 3 and at 4°C from day 7.

Figure 4: Gel of RNA Stored at Various Temperatures



Lanes 1&2: Room temperature for 28 days
Lanes 3&4: 4°C for 28 days
Lanes 5&6: -20°C for 28 days
RNA stored at room temperature for 28 days shows degradation.

Figure 5: Gel of RNA Stored at -20°C



RNA stored at -20°C showed the RNA integrity remaining unchanged. Similar results were obtained for storage at 4°C and for freeze-thaws from -20°C and -80°C (data not shown).

SUMMARY AND CONCLUSIONS

To demonstrate purified RNA storage stability at different conditions, tissue RNA was extracted then normalized to a concentration of 324 µg/mL with a RIN number of 8.1. The aliquoted RNA was stored under different condition and was assessed by Agilent gels, RIN, rRNA ratio, and RNA concentration. The RNA aliquots stored at -20°C showed stable RNA integrity, rRNA ratio, and RNA concentration for up to 28 days. RNA aliquots used for up to 10 freeze-thaw cycles with storage at -20°C or -80°C also showed stable results for all parameters tested. When the RNA aliquots were stored at 4°C, RNA integrity and rRNA ratio were not affected but the volume started to evaporate at day 7. When the RNA aliquots were stored at room temperature, the volume started to evaporate on day 3, the ribosomal RNA ratio started to decline on day 7, and RNA integrity started to decline on day 14.

Test Condition	Results
-20°C	Stable for at least one month
-80°C	Stable for at least one month
4°C	Stable for 14 days
Room temperature	Stable for 2 days
Freeze-Thaw	Stable up to 10 cycles

Conclusions:

- The quality of extracted RNA primarily depends on the quality of the original material.
- Extracted RNA stored at -20°C and -80°C was of good quality, and the RNA was stable for up to 10 freeze-thaw cycles.
- Extracted RNA can be stored at 4°C for 14 days without degradation. Evaporation may occur during this time.
- Extracted RNA can be stored safely at room temperature for 2 days without degradation.

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