

## GenTegra RNA Preserves the Quality and Integrity of Purified RNA During Ambient Temperature Transport

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### Abstract

GenTegra® RNA is a groundbreaking technology for the stabilization, transport and storage of purified RNA. GenTegra is a water-soluble, inert chemical matrix that inactivates trace RNase in the liquid phase, providing an added level of RNA stability during sample handling, with or without ice. In the dry-state, GenTegra preserves RNA integrity by protecting samples from hydrolysis and oxidation, imparts thermal stability at temperatures ranging from -80°C to 76°C/169°F and ensures quantitative recovery of RNA upon rehydration. Previously, the GenTegra RNA format has been validated for 1-20µg of RNA in a volume of 20-50µl<sup>1</sup>. In this study, we report that GenTegra RNA preserves the integrity of 50µg purified RNA samples derived from various human, animal and plant cells at ambient temperature during storage and transport in the dry state, with no effect on RNA quality or integrity.

### Introduction

BioChain Institute, Inc. is a company with integrated tools for drug target validation and diagnostic assay development. With more than 5,000 ready-to-use products, including reagents and kits for nucleic acid extraction, purification and analysis, as well as custom-designed products and services, they are enabling biomedical researchers to conduct their experiments and develop their assays with unprecedented efficiency. RNA samples are frequently used in Biochain's work-flow. Once purified from cells or tissue, RNA stability must be maintained to avoid the loss of valuable samples. Preventing degradation of RNA, which is primarily induced by RNases that co-purify with nucleic acids or are introduced from the environment, is a major challenge, as is protecting purified RNA during long-term storage and transport. Biochain faces

these challenges when working with RNA purified from diverse cell sources. The use of purified RNA is an important part of the BioChain's ongoing research to understand the biology and pathogenesis of cancer and other disease processes. RNA samples with a mass of 50µg are often transported to and from their various collaborators.

Shipping delays between locations in the United States and foreign countries can result in loss of RNA sample integrity. Even pristine RNA samples packed in dry ice and shipped according to specifications may be ruined due to unforeseen shipping delays or other problems. To address the difficulties faced by scientists working with purified RNA specimens, GenVault has developed GenTegra® RNA, an inert, water-soluble, chemical matrix, which provides protection from oxidation and hydrolysis, conveys thermal stability at temperatures ranging from -80°C to 76°C/169°F that may occur during shipping, and assures quantitative recovery of RNA samples. Here, we demonstrate that storage of 50µg of purified RNA in GenTegra RNA for three weeks at ambient temperature, simulating shipping delays, has no effect on RNA integrity, making GenTegra a safe, convenient method of stabilization, transport and storage of purified RNA.

### Materials and Methods

#### Purification, Application, Storage and Recovery

Eight samples of total RNA were purified from human lung and pancreas, rat and mouse pancreas, wheat, Raji, and placenta using Biochain's Dr. P kit. See Table 1 for sample identification. Two 50µg aliquots were purified from each sample type, and the purified RNA samples were labeled G1 - G8 (for GenTegra RNA samples) and F1 - F8 (for frozen samples at -80°C), respectively.

Table 1. Identification of RNA samples derived from eight tissue types.

Sample ID	Tissue Type	Lot ID
1	Human Lung	A511335
2	Human Lung	A808157
3	Human Pancreas	B103010
4	Rat Pancreas	B211030
5	Mouse Pancreas	B204036
6	Wheat	B304089
7	Raji Cells	A802170
8	Placenta	B209047

RNA specimens were diluted to a concentration of 1µg/µl and 50µl aliquots (50µg) were either applied to GenTegra RNA tubes immediately after isolation and quantitation (for dry phase storage at 25°C), or stored overnight at -80°C prior to application to GenTegra RNA tubes. An aliquot of each RNA sample was stored at -80°C as a frozen control. 50µg aliquots of each sample were applied to GenTegra tubes. Following application, one set of RNA samples was dried overnight (16hr) in a FastDryer (GenVault, Carlsbad, CA) according to the manufacturer's instructions. Samples were then stored in the dry state for three weeks at 25°C. An identical set of samples was stored at -80°C as frozen controls. Following the dry storage period, the samples were rehydrated in 50µl of molecular-grade DEPC water according to the manufacturer's instructions.

### RNA Quantitation and Gel Electrophoresis

RNA was quantified using a NanoDrop 1000 (ThermoScientific, Wilmington, DE). Information about RNA integrity was gathered from an electrophoretic trace and a RNA Integrity Number (RIN) was obtained using the RNA 6000 Nano Chip on the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA) according to the manufacturer's protocol. RIN values are given on a scale of 1 to 10, with 1 being the most degraded profile and 10 being the most intact<sup>2</sup>. To further assess the quality of RNA after application to GenTegra RNA tubes, as compared to frozen controls held at -80°C, RNA (2µg) was run on a 1% agarose gel.

### Results

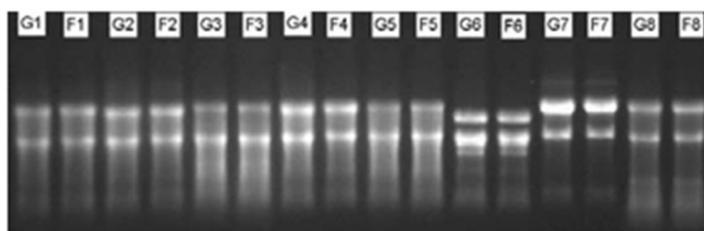
At the end of the three week storage period, RNA samples were rehydrated in 50µl of molecular-grade

DEPC water and quantitated via NanoDrop 1000. No loss of RNA was observed in samples stored in GenTegra RNA tubes. A slight increase in RNA concentration was observed with samples recovered from GenTegra RNA, with an average recovery yield of 118%. This increase might result from rehydration of the dried RNA in a slightly smaller volume of water compared with the input volume. The average recovery yield for samples stored at -80°C was 99.8%. Table 2 details the original and recovery concentration for the 50µg RNA samples.

Table 2. Concentration of RNA stored in the dry state at 25°C for three weeks in the presence of GenTegra or frozen at -80°C.

Sample ID	Tissue Type	Starting Conc. (ng/µl)	Conc. Following 3 wk storage (ng/µl)	
			GenTegra	-80°C
1	Human Lung	960.32	1398.82	956.14
2	Human Lung	902.24	1020.88	901.29
3	Human Pancreas	879.97	1017.77	866.45
4	Rat Pancreas	1052.52	1203.8	1049.03
5	Mouse Pancreas	1077.11	1270.24	1073.46
6	Wheat	1003.48	1065.31	1009.13
7	Raji Cells	942.52	1100.49	943.1
8	Placenta	951.36	1105.53	952.74

RNA aliquots in the dried state at 25°C for three weeks in the presence of GenTegra RNA were compared to control samples stored at -80°C by gel assessment for RNA quality (Figure 1). No difference in integrity was observed for any of the samples stored at 25°C in the presence of GenTegra (G1-G8) and the frozen controls (F1-F8). The RIN of samples F1-F8 and G1-G8 are summarized in Table 3, with the exception of sample 6 (wheat), where a RIN could not be determined due to degradation of the starting sample which was evident in the electropherogram.

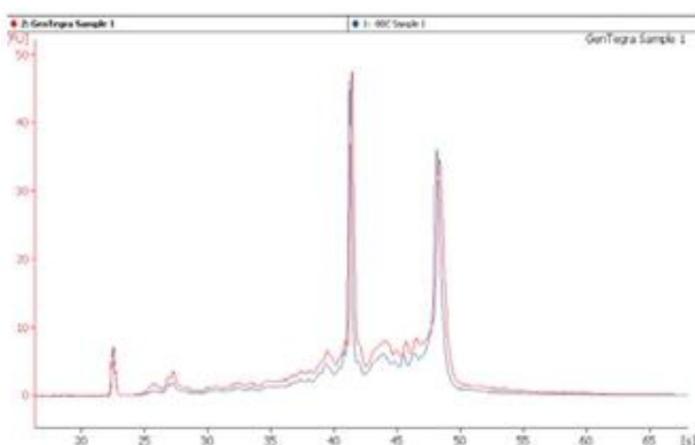


**Figure 1.** Gel analysis of RNA samples stored in the presence of GenTegra RNA (G1-8) in the dry state for 3 wks at 25°C and controls stored at -80°C (F1-8).

**Table 3.** RIN of samples stored in the dry state for 3 wks at 25°C as compared to frozen controls at -80°C.

Sample ID	Tissue Type	Starting RIN	RIN Following 3 wk storage period	
			GenTegra	-80°C
1	Human Lung	7.5	7.5	8
2	Human Lung	8	7.9	8.7
3	Human Pancreas	6.5	6.3	6.9
4	Rat Pancreas	7.4	7.6	8.2
5	Mouse Pancreas	5.5	5.5	5.8
6	Wheat	N/A	N/A	N/A
7	Raji cells	10	10	10
8	Placenta	8.1	8.2	8.7

Complete electropherograms were generated by the Agilent Bioanalyzer for all eight RNA sample sets. A representative electropherogram for sample 1 (human lung) is shown in Figure 2.



**Figure 2.** Agilent BioAnalyzer Data for human lung: samples G1

The data for samples stored in GenTegra RNA (red lines) are super-imposed over control samples stored at -80°C (blue lines). After 3 weeks in the GenTegra RNA system at ambient temperatures, there is no significant change in the RIN values of the samples. Samples stored at -80°C for 3 weeks showed slight increases in the RIN value for the samples, but this is within the assay's 10% CV for reproducibility.

## Conclusions

GenTegra RNA is a water-soluble inert chemical matrix that preserves RNA integrity in the dry-state for storage and transport. In the present study, we examined the use of GenTegra RNA for ambient temperature storage of 50µg of total RNA purified from various cells and tissues. The goal of this three week study was to simulate the effects of ambient temperature transport Pancreas in the case of an extreme shipping delay. Here, we have shown that the integrity of 50µg samples of RNA stored in the dry-state, at 25°C, is preserved in the presence of GenTegra RNA for three weeks. Integrity was assessed by gel analysis, NanoDrop quantitation and Agilent Bioanalyzer analysis. Using GenTegra RNA for shipping eliminates the need for dry ice or overnight shipping and eliminates concern about RNA degradation due to shipping delays.

## References

1. IntegenX GenTegra RNA User Manual, Version 3, January, 2010.
2. RNA Integrity Number (RIN) - Standardization of RNA Quality Control. Publication Number: 5988-8322EN, 2006.