

STABILIZATION AND SHIPPING OF RNA AT AMBIENT TEMPERATURES

A cumulative summary of research investigations of RNAsable as a preservative of RNA at ambient temperatures.

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Executive Summary

RNA is a very labile material hence requiring special handling to maintain/retain sample quality for studies involving gene transcription and regulation. The typical method of storing and handling RNA samples is by cold-freezing at ultra low temperatures (-80C or LN2), so as to preserve sample integrity. Shipping biological samples from collection (field, clinics, etc) sites to the lab for RNA extraction and analysis is often a very costly exercise since it requires the use of bulky materials such as styrofoam boxes and dry-ice. Such bulky packaging often results in expensive shipping charges (see Table 1) even for local shipments. Regardless of cost, RNA samples are too labile to ship otherwise, hence will continue to require bulky packaging and high shipping costs... Or should it?

Table 1: Economics of biostabilization – shipping RNA on dry ice versus with RNastable

Shipment type	Germany	Australia	India	Japan
Dry Ice, FedEx*	\$ 178	\$ 202	\$ 205	\$ 172
Ambient, USPS	< \$5	< \$5	< \$5	< \$5
Cost savings	> \$173	> \$197	> \$200	> \$167
*1-10 samples with 10lb of dry ice				

Biomatrix has formulated an ambient temperature preservative for purified RNA samples for long-term storage or shipping from field to lab. RNastable offers a cost-effective alternative to cold-packing and shipping (see Table 1) because it protects RNA at ambient conditions requiring only a shipping envelope to transport. It can also be used to concentrate RNA for analysis.

This report documents the current validation studies that have been conducted to evaluate and validate the use of RNastable as a preservative for RNA, without compromising quality or integrity.

Introduction

RNastable was launched in 2009 as a dry-matrix formulation for ambient temperature based long-term preservation, shipping and storage of purified total RNA. The longest recorded storage of RNA in RNastable is 29 months at room temperature and at 45⁰C.¹ RNastable is manufactured for sale in 1.5ml tubes or 96-well plate formats (see Figure 1).



Figure 1: RNastable tubes and 96-well formats

¹ Accelerated aging study: 29months at 45C ~ 12 years.

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RNA stabilization product (RNASTable®)

RNASTABLE COMPATIBILITY WITH MICROARRAY EXPRESSION ANALYSIS

Hernandez and co-workers² at The Scripps Research Institute investigated the preservation of RNA at room temperature (RNASTable) versus the traditional ultra-cold storage (-80C) preservation, including shipping RNA samples to a Core Facility for microarray gene expression analysis. RNA samples were mixed and stored in 3 ways: (1) dry at room temperature in RNASTable, (2) frozen at -80C, or (3) initially preserved in RNASTable and then stored at -80C. Tables 1 and 2 display the results of the study.

Table 1: Recovery and integrity of RNA stored in RNASTable

RNA Sample	Storage Condition	ng/uL	OD _{260/280}	RIN Score
Human Liver total RNA	RT for 4 weeks (RNASTable)	219 ± 10	2.02 ± 0.03	9.70 ± 0.00
	-80C for 4 weeks (Rnase-free water)	217 ± 9	2.05 ± 0.01	9.73 ± 0.06
Human Kidney total RNA	RT for 1 week; -80C for 4 weeks (RNASTable)	243 ± 15	2.00 ± 0.05	8.08 ± 0.15
	-80C for 5 weeks (Rnase-free water)	296 ± 7	1.98 ± 0.01	7.98 ± 0.15

Table 2: Glyco_v3 microarray analysis of human liver total RNA stored in RNASTable

Storage Condition	Background	Noise	% Present	GADPH (3'/5' ratio)	BETA-actin (3'/5' ratio)
RNASTable (RT for 4 weeks)	37.3 ± 1.6	1.6 ± 0.2	59.3 ± 1.0	1.14 ± 0.06	4.22 ± 0.71
Control (-80C for 4 weeks)	37.3 ± 1.6	1.7 ± 0.1	59.4 ± 1.3	1.10 ± 0.06	4.10 ± 0.20

**Glyco_v3 microarray is a custom GeneChip expression array built by Affymetrix for the Consortium of Functional Glycomics; n = 3.*

In conclusion, the authors found that RNASTable provided comparable stabilization of RNA as cold-stored (-80°C) samples, in quality and yield. The protective properties of RNASTable were unaffected in low temperature conditions, and concluded that “... based on our results, we have found that room temperature storage of RNA in RNASTable for ≤4 weeks is suitable for samples destined for microarray analysis, and provides an alternative to conventional cold storage and transport procedures currently used.”

² Hernandez, G.R., Mondala, T.S., and Head, S.R., *Assessing a novel room temperature RNA storage medium for compatibility in microarray gene expression analysis*, *Biotechniques*, **2009**, 47: 667-670.

RNA LEAF EXTRACTS

In Professor Virginia Walbot's lab (Stanford U), maize leaf RNA were extracted and stored both at room temperature (in RNAsstable[®]), and in ultra low temperature (-80°C) conditions for 8 weeks.³ Analysis of total RNA quality was performed using Bioanalyzer for samples stored under both conditions (see Figure 2). The results clearly indicate that RNAsstable is a good matrix to stabilize and store purified RNA samples with equal performance as cold-stored samples.

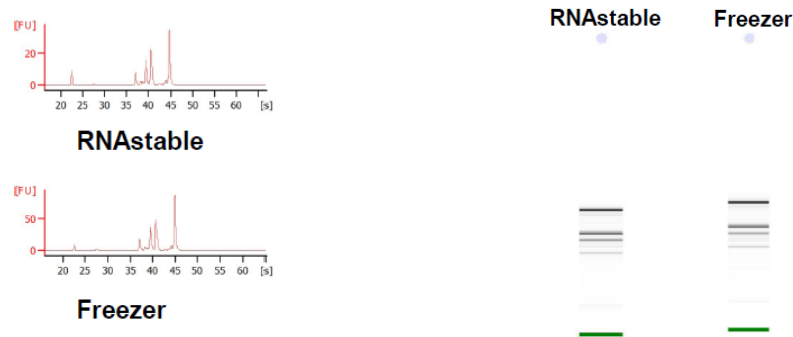


Figure 2: Bioanalyzer evaluation of the quality of total RNA stored in RNAsstable (room temperature) or in freezer (-80°C) shows no difference between both storage options.

TOTAL RNA AND MIRNA ANALYSIS

Professor David Hirschberg's lab (Columbia U⁴) evaluated the amount of total RNA recovered (ng/μL) and sample quality (RIN scores) of four RNA samples stored in RNAsstable (room temperature) as well as in ultra low temperature (-80°C) freezer. The results displayed in Figure 3 show no difference in the measured metrics for samples stored under both conditions.

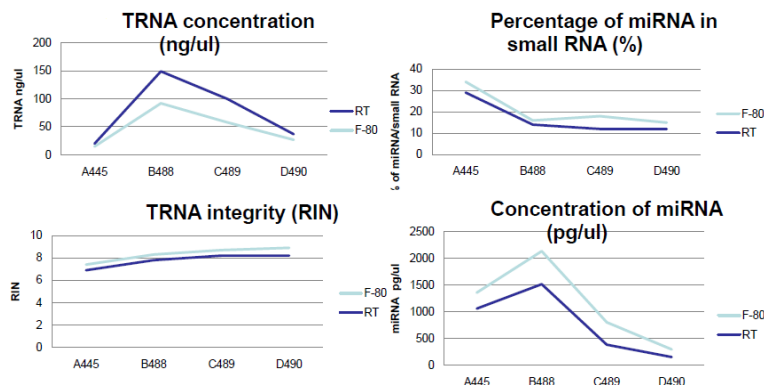


Figure 3: Measured yield and sample quality of total RNA and miRNA in samples stored in RNAsstable[®] (room temperature; —) and at -80°C (—)

³ Unpublished data. Reproduced with permission.

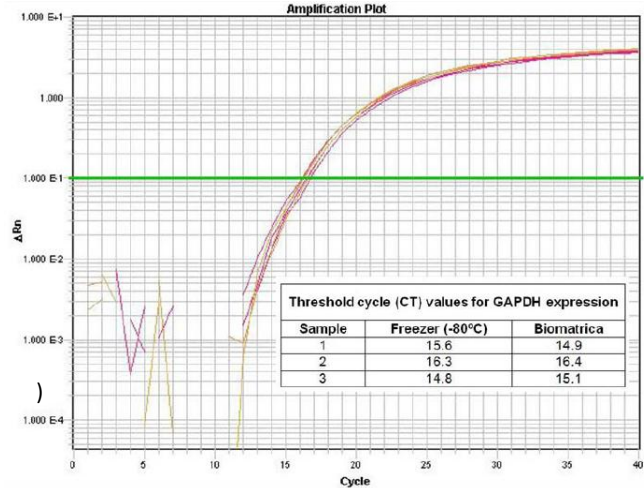
⁴ Research was conducted while at Stanford University.

RNA SAMPLES FROM SKIN TISSUE

At the Kwok laboratory (UCSF), 3 RNA samples extracted from skin tissue were stored in RNastable (at room temperature) as well as in an ultra low temperature freezer (at -80°C) for 11 days. The samples were recovered and analyzed for sample quality (RIN scores) and use in downstream experiments (PCR). The results (see Figure 4) conclude that RNA samples can be safely stored in RNastable at room temperature without degradation, and used in downstream analysis such as PCR or expression profiling.

Sample	Before Storage	Freezer (-80°C)	Biomatrix
1	9.1	8.9	9.2
2	7.4	7.6	7.6
3	8.7	8.2	8.8

(a)



(b)

Figure 4: (a) RIN scores, and (b) qPCR results for RNA samples recovered after 11 days storage at room temperature (RNastable[®]) and at -80°C (freezer).

RNA PRESERVATION IN COLON CANCER TISSUES MEASURED BY LCM

Dr John Gillespie⁵ investigated the efficacy of RNA preservation in colon cancer tissues by comparing untreated slides and slides treated with RNastable using the protocol described below (Figure 5). In the study, 8μ of frozen colon cancer tissue sections were cut onto charged slides stained with hematoxylin and eosin. After staining, slides were either treated with RNastable (to preserve RNA in samples), RNase inhibitor, or untreated (no preservative), and stored at different intervals ($t = 0, 48\text{h}$ and 7 days), at room temperature. The quality and quantity of the RNA extracted from these tissues were determined using the Bioanalyzer RIN scores and reverse transcription qPCR for actin-3':M ratio (see Figure 6).

The authors concluded that (a) tissue treatment with RNastable or RNase inhibitors did not impact micro-dissection, (b) there was no measurable difference in the quality of RNA between untreated and RNase treated samples over time as RNase inhibitors did not improve RNA preservation in tissue, and (c) nucleic acid preservative-treated slides with RNastable, retained excellent quality RNA up to 7 days and very good quality after 28 days versus untreated slides which showed poor quality after only 48 hrs.

⁵ Formerly at the National Cancer Institute (NCI); data reproduced with permission.

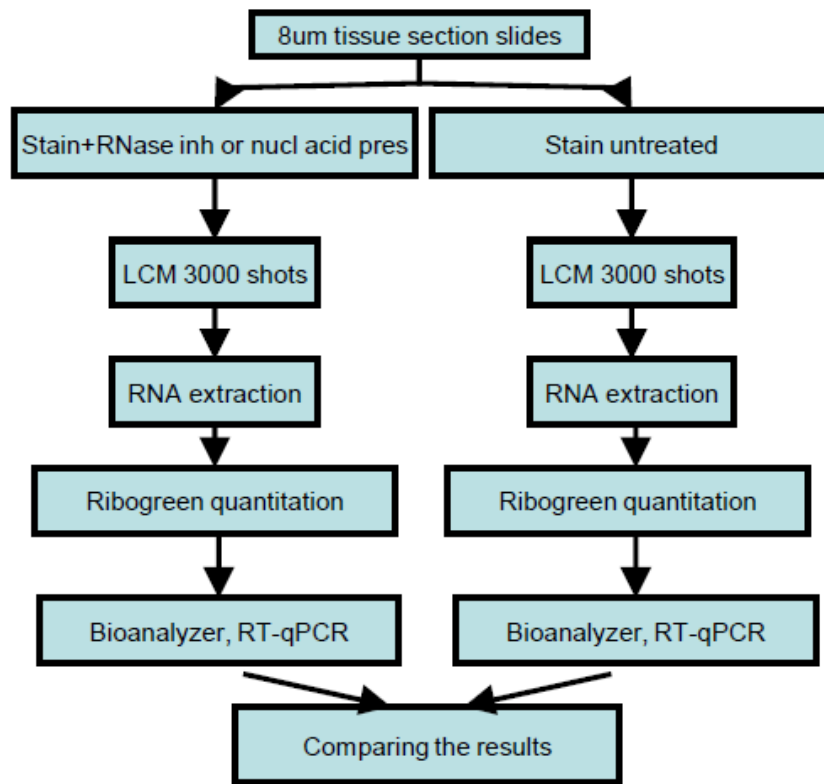


Figure 5: Schematic workflow used to evaluate RNA stabilization in colon cancer tissue samples.

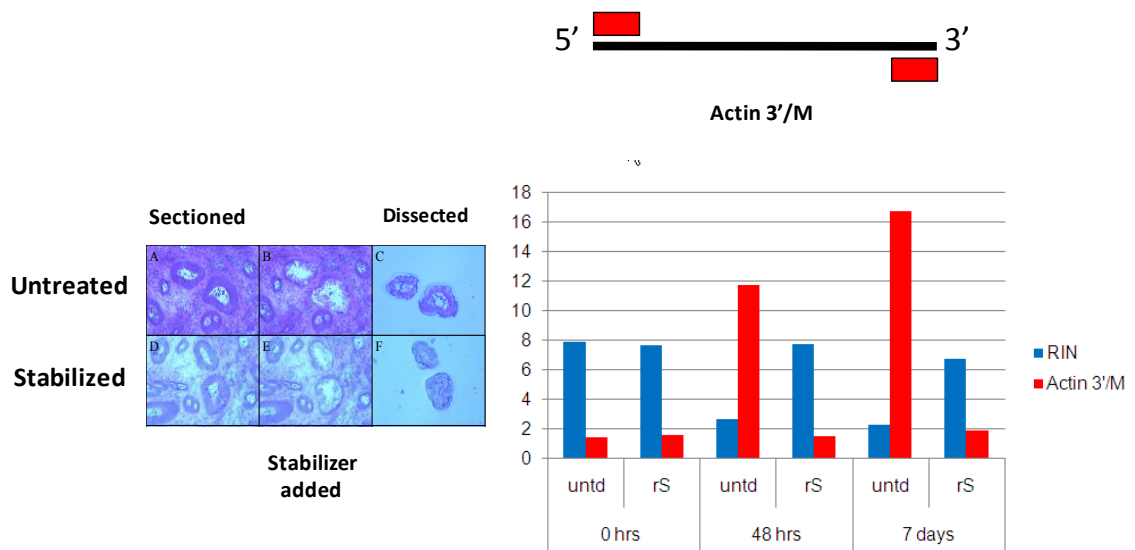


Figure 6: Results of LCM analysis of RNA preservation in tissue by RNastable (treated/stabilized) versus untreated (no preservative), based on RIN scores (■) and actin-3':M ratio (■) measurements.

RNA SEQUENCING ANALYSIS OF VIRAL RNA FROM *PLASMODIUM FALCIPARUM*

Dr Grant Hill-Cawthorne⁶ has employed next gen sequencing-based analysis (RNA-Seq) of viral RNA extracted from the malaria parasite – *plasmodium falciparum* – that is untreated (unprotected) or treated (preserved) with RNAsable at room temperature. The purified RNA extract was coated with RNAsable and shipped from the UK to Saudi Arabia with shipping temperatures varying as high as 45°C. After rehydrating and analysis, results of the quality mapping (Figure 7) showed that for the RNAsable-preserved samples, RNA reverse transcribed into cDNA well enough and in long enough fragments (up to 90bps) for excellent library making and sequencing. It is also worth mentioning that there was no interference of the RNAsable matrix in the sequencing experiment.

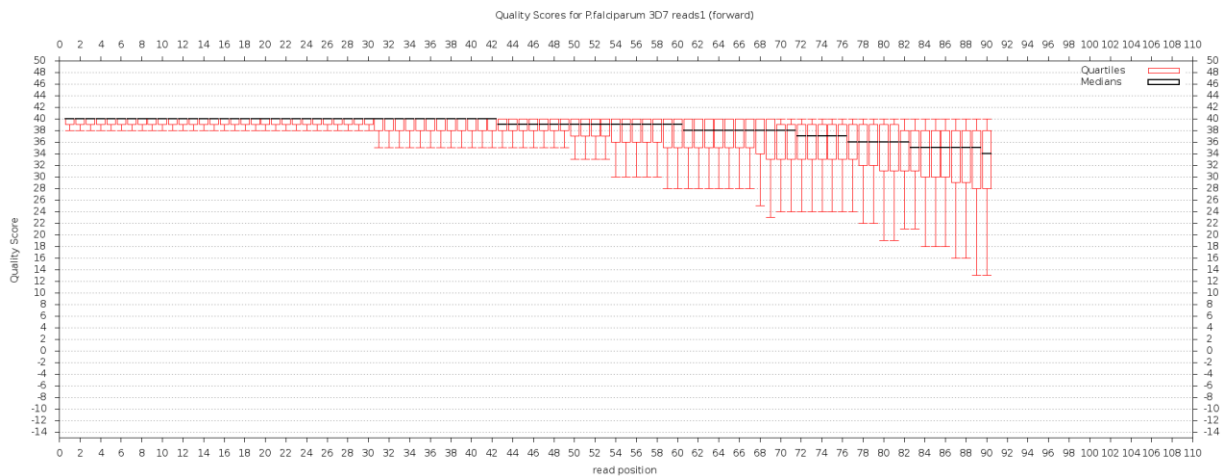


Figure 7: Quality mapping of transcription of RNA into cDNA (forward and reverse strand) measured by Illumina's GAII sequencer.

STABILIZING FPET RNA IN CANCER SAMPLES

At Genomic Health, an evaluation of the stabilization of RNA extracted from two different cancer cell lines – breast and colon - was conducted over a 12-month period with RNAsable versus -20C storage. The primary goal of the study was to evaluate the ability of RNAsable to preserve the gene expression profiles for FPET RNA over the course of 12 months. For the study, 5 different RNA samples were selected for storage at -20C and at room temperature (in RNAsable) – Stratagene's UHR RNA (1), breast FPET RNA (2), and colon FPET RNA samples (2).⁷

About 1µg of RNA samples was added to wells in a 96-well RNAsable plate and dried-down as per protocol and rehydrated at different time points: t=0, 1week, 3months, 6months and 12months. 96 RT-qPCR assays were tested in quadruplicate at each time point on a Roche

⁶ Pathogen Genomics Laboratory, King Abdullah University of Science and Technology, Thuwal Kingdom of Saudi Arabia,

⁷ Study performed at Genomic Health, 101 Galveston Dr., Redwood City, CA 94063.

LightCycler 480. Gene expression values (Cp) were compared across each time point, and quality score values assigned using proprietary scoring matrix at GH was used to assess the impact of RNAsable on stored RNA samples. Results of the study (Cp values) are shown in Figures 8 and 9, for colon and breast FPET RNA samples, respectively.

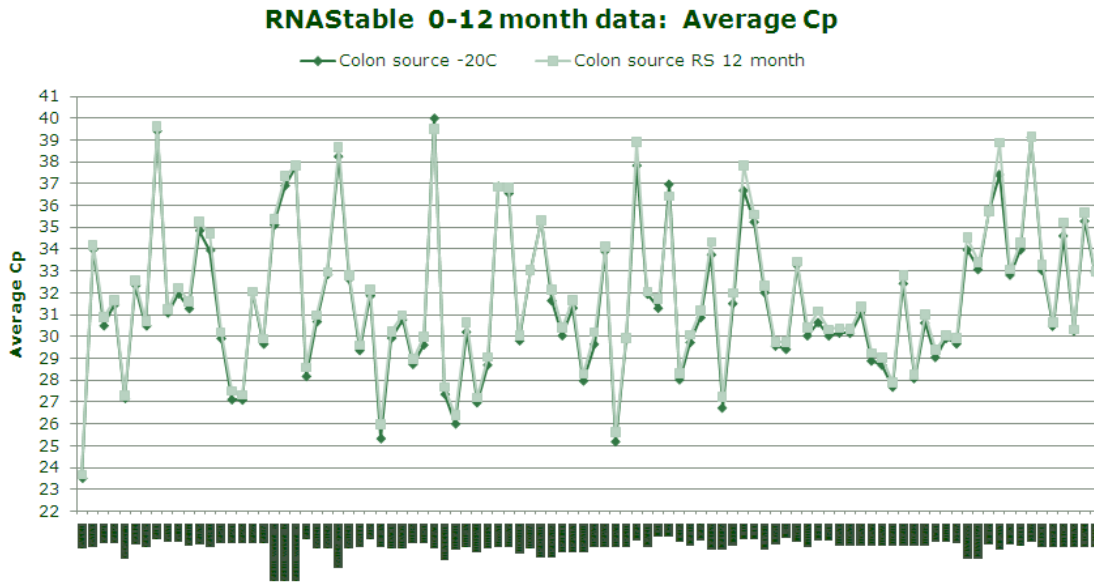


Figure 8: Comparison of FPET RNA in colon samples stored frozen (-20C) and at room temperature (RNAsable) for 12 months.

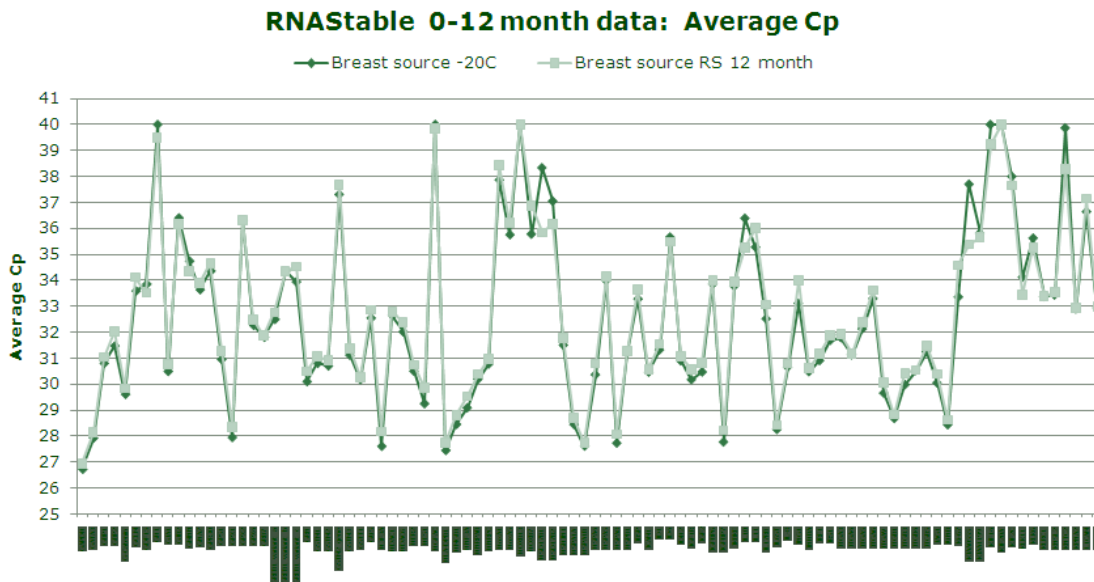


Figure 9: Comparison of FPET RNA in breast samples stored frozen (-20C) and at room temperature (RNAsable) for 12 months.

In summary, the study found that RNASTable is able to preserve the gene expression profiles of qPCR assays in both intact RNA as well as FPET RNA for up to one year, and there was no discernible difference in performance against -20C stored/preserved RNA samples across all sample sources.

References

ARTICLES

1. S. Ohgi, L. Coulon, R. Muller, J. Muller-Cohn and O. Clement, [Stabilizing RNA at room temperature in RNASTable](#), *Biotechniques*, **2010**, 48(6), 470.
2. Gilberto E. Hernandez, Tony S. Mondala, and Steven R. Head, [Assessing a novel room-temperature RNA storage medium for compatibility in microarray gene expression analysis](#), *Biotechniques*, **2009**, 47: 667-670.
3. Ohgi, Sharron. Making RNA more durable at room temperature. *Genetic Engineering and Biotechnology News*, **2007**, 27, 18.

APPLICATION NOTES

4. Rhada Gopal & Fernando P. Monroy, [Improved QPCR Analysis Following Storage in RNASTable[®]](#)
5. Vincent Funari, [Microarray Analysis of RNA in RNASTable[®]](#)
6. [microRNA \(miRNA\) Stabilization in RNASTable[®]](#)
7. [RNA Stabilization at Room Temperature in RNASTable[®]](#)
8. [Stabilization of poly\(A\) mRNA transcripts in RNASTable](#)

List of organizations recommending RNASTable for shipping RNA samples

1. **Phalanx Biotech**
<http://www.phalanxbiotech.com/Products/rnastable.html>
2. **DNA Array Core Facility/CFG at The Scripps Research Institute**
<http://www.functionalglycomics.org/static/consortium/resources/resourcecoree2.shtml>
3. **Empire Genomics**
<http://www.empiregenomics.com/main/resources/faq/167-prepare-rna-sample-for-mailing>
4. **ArrayStar**
<http://www.arraystar.com/manage/UploadFile/200992722532290.pdf>
5. **Microarray Facility at The Hospital For Sick Kids**
http://www.tcag.ca/Microarray/09_10_RNAAnalysisRequestForm.pdf