# RNA Stabilization User Guide

GENEWIZ®
A Brooks Life Sciences Company

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**FOR RESEARCH USE ONLY**

## Product Specifications

- Quantitative recovery of up to 20 µg of RNA
- Quality of stabilized RNA is comparable to input RNA
- Recovery in a volume of 20 to 50 µL
- Compatible with samples containing trace RNase activity
- Increased stability of dissolved RNA for up to 100 hours at room temperature (21 to 25°C) upon application
- Increased stability for up to 8 hours following rehydration of dried RNA, across up to 5 cycles
- Compatible with RNA from cell lines, blood, PAXgene® tubes, fresh and frozen tissue, and FFPE tissue
- Compatible with RNA purified using standard kits and protocols (e.g. Invitrogen®, Ambion®, QIAGEN®, TRIZOL®)
- Compatible with all common storage buffers, including water, TE, EDTA, and citrate (Note: TE buffer is not recommended for samples that may be subjected to elevated temperatures during transport)
- Use in downstream applications without further purification; does not inhibit qPCR or expression profiling
- Thermal stability from -80°C to 76°C during transport
    - Exceeds military specifications (-60°C to 71°C)
    - Exceeds FedEx® specifications (-51°C to 60°C)

## Storage and Transport

- Store and transport at ambient temperature

## Simplified Workflow

Add RNA to RNA Stabilization tubes

↓

Dry for storage or shipping at ambient temperature

↓

To recover, reconstitute with molecular biology grade water

↓

RNA is ready for use

## Protocols

## Stabilization of RNA

1. Prepare ≤20 µg of purified RNA in a volume of 20-50 µL. A minimum of 20 µL is necessary for sufficient mixing of RNA and the stabilization matrix. For concentrated samples, dilute with molecular biology grade water prior to addition.
2. Add purified RNA to the bottom of the RNA Stabilization tube. The stabilization matrix is supplied as a coating at the bottom of the tube.
3. Incubate for 5 minutes at room temperature (21-25°C).
4. Mix by pipetting up and down 10 times to solubilize and mix in the matrix.
5. For continued use of RNA in dissolved form, proceed to the protocol on page 6.
6. For transport or long-term storage of RNA, proceed to the drying protocol on page 7.

## Using Stabilized RNA in Dissolved Form

The chemical formulation in RNA Stabilization tubes preserves RNA by inactivating trace nucleases and protecting against oxidation.

1. Apply RNA to the RNA Stabilization tubes according to the protocol on page 5.
2. Keep stabilized RNA in dissolved form when aliquots will be used within 100 hours. Stabilized RNA may be used at room temperature (21 to 25°C) or on ice.
3. After 100 hours, dry the sample down (see page 7) or store at -80°C.

## Drying Stabilized RNA

1. Remove the caps and dry with one of the following methods.
    - Air dry in a biosafety hood for 18-24 hours*.
    - Use a SpeedVac® for 2-4 hours* at room temperature. See page 8 for further instructions.
    - Vacuum desiccate for 3-4 hours* at room temperature. See page 8 for further instructions.

    *Actual drying times may vary by total volume and RNA concentration.
2. Confirm tubes are completely dry by visual inspection.
3. Cap the tubes and store at room temperature.

## Drying RNA Using a SpeedVac

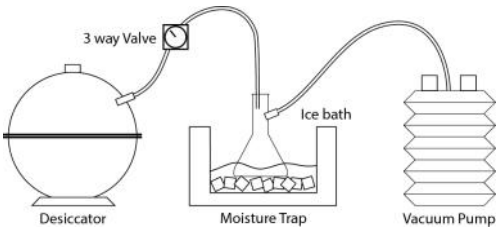
A SpeedVac may be used to dry up to 50 µL of RNA. Drying times are approximate and may need to be modified based on the system. Ensure that tubes are completely dry by visual inspection.

1. Place tubes with caps off in the SpeedVac.
2. Ensure that the temperature setting does not exceed 30°C.
3. Dry tubes for approximately 2-4 hours.
4. Following drying, cap tubes and store or transport at ambient temperature.

## Drying RNA Using a Vacuum Desiccator

A vacuum desiccator may be used to dry up to 50 µL of RNA. Drying time is approximate and may need to be modified based on the system. The setup consists of a vacuum desiccator, vacuum pump, a vapor trap, assorted tubing, and a small ice bath (see figure below). Ensure that tubes are completely dry by visual inspection.

1. Place tubes in a rack and place the rack in the desiccator.
2. Close the desiccator and turn on the vacuum pump.
3. Dry tubes for approximately 3-4 hours.
4. Following drying, cap tubes and store or transport at ambient temperature.



## Recovery of Dried RNA

1. Add a volume of molecular biology grade water equivalent to the input volume.
2. Incubate the tubes at room temperature (21 to 25°C) for 10 minutes. **Do not attempt to recover RNA on ice.**
3. Pipette up and down 10 times to solubilize the RNA. Alternately, tubes may be capped, vortexed for 10 seconds, and centrifuged briefly.
4. RNA is ready for use in QC or downstream applications.
5. Recovered RNA may be used for up to **8 hours in dissolved form** at room temperature (21 to 25°C) or on ice.
6. After 8 hours, dry down or store recovered RNA at -80°C.

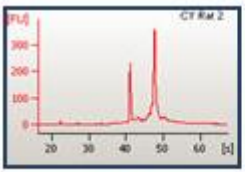
## Product Information

| RNA Stabilization 2 mL Screw-Cap Tubes | |
|---|---|
| Catalog # | GTR5025-GW |
| Tube Volume | 2 mL |
| Input RNA Volume | 20-50 µL |
| Input RNA Amount | ≤20 µg |
| Input RNA Concentration | ≤1 µg/µL |
| Recovery Volume | Equivalent to input volume |
| Recovery Amount | Up to input amount |
| Recovery Concentration | Up to input concentration |
| Drying Method | Air dry, SpeedVac, or vacuum desiccator |

## Technical Information

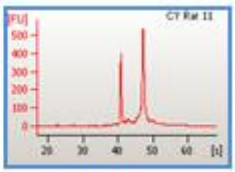
## Storage of Rat Liver RNA

RNA Stabilization Tubes 25°C




RIN 10

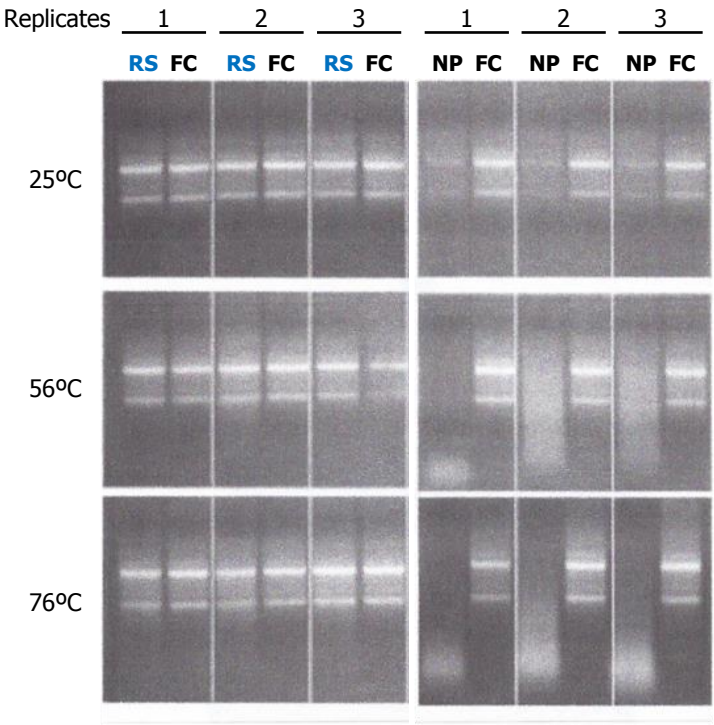
Frozen Control –80°C




RIN 10

Quality and integrity of RNA stored in RNA Stabilization tubes is identical to frozen RNA. Total RNA (20 µg) purified from rat liver was stored in the dry state at 25°C for 30 days and compared with a control stored frozen at -80°C. RNA integrity was examined using a 0.8% agarose gel stained with ethidium bromide and using an Agilent® Bioanalyzer.

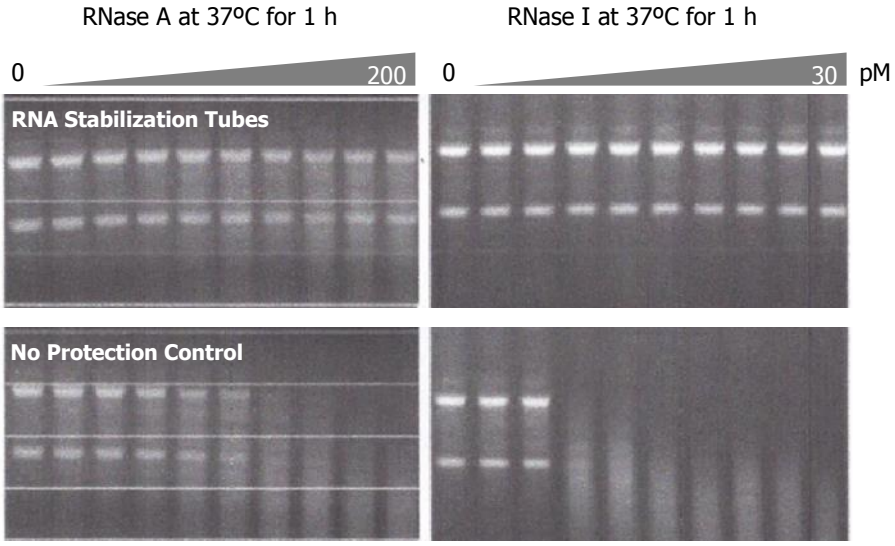
## Storage of PAXgene RNA




**RS** = RNA Stabilization tubes
**FC** = Frozen control (-80ºC)
**NP** = No protection control

The integrity of PAXgene RNA stored in RNA Stabilization tubes is equivalent to RNA stored frozen. RNA was purified from individual PAXgene tubes and split into multiple aliquots. One aliquot of each sample was stored at -80°C (FC). Others were added to RNA Stabilization tubes and dried (RS) or left unprotected (NP) and incubated at 25°C, 56°C, or 76°C for 30 days.

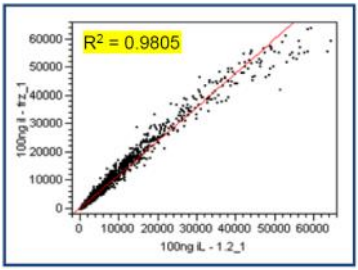
## Stabilized RNA in the Presence of Trace RNase



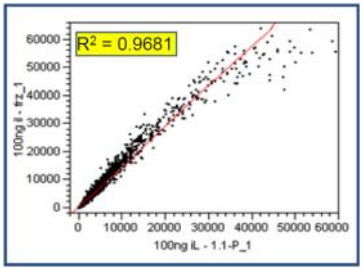

RNA integrity is maintained in RNA Stabilization tubes with increasing amounts of added RNase. HeLa cell RNA (5 µg) was incubated with RNase A or RNase I at 37ºC for 1 hour with or without RNA Stabilization.

## Illumina Expression Profiling with Recovered RNA

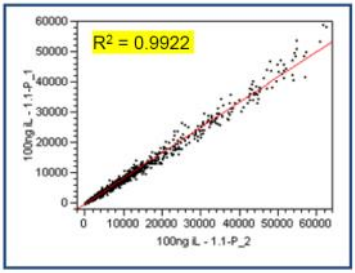
Replicate 1:
Frozen vs RNA Stabilization




Replicate 2:
Frozen vs RNA Stabilization




RNA Stabilization Replicate 1
vs
RNA Stabilization Replicate 2

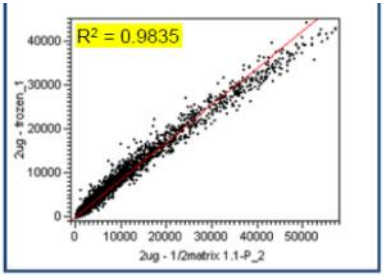



Expression profiling of RNA using the Illumina® HumanHT-12 Expression BeadChip Kit. Replicate RNA samples purified from HeLa cells (20 µg) were stored in the dry state for 2 weeks at 25°C with RNA Stabilization and compared with a control stored at -80°C. Gene expression profiles between the two conditions and replicates show strong correlation, suggesting comparable input RNA quality.

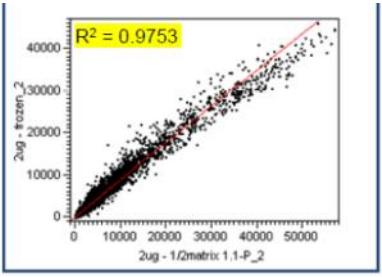
# Affymetrix Expression Profiling with Recovered RNA

**2 µg Protocol**

Replicate 1:
Frozen vs RNA Stabilization

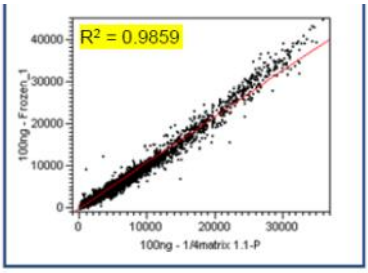



Replicate 2:
Frozen vs RNA Stabilization

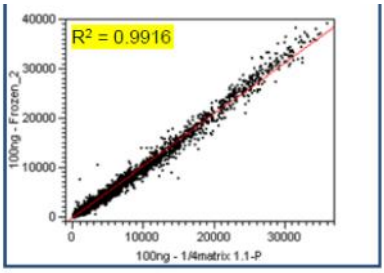



**100 ng (Low Mass) Protocol**

Replicate 1:
Frozen vs RNA Stabilization




Replicate 2:
Frozen vs RNA Stabilization



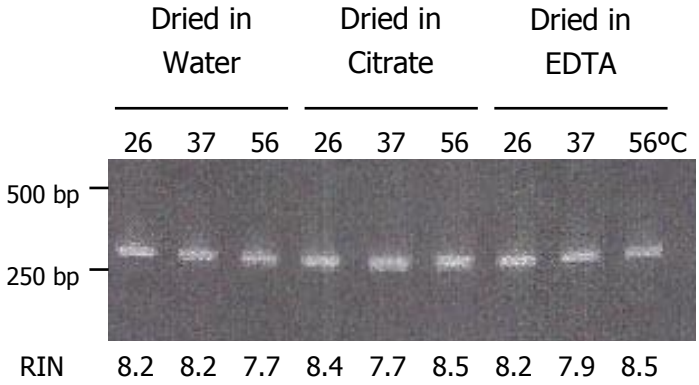

Expression profiling of RNA using the Affymetrix® GeneChip™ Human Genome U133 Plus 2.0 Array. Replicate RNA samples purified from HeLa cells (20 µg) were stored in the dry state for 2 weeks at 25°C with RNA Stabilization and compared with a control stored at -80°C. Gene expression profiles between the two conditions show strong correlation, suggesting comparable input RNA quality.

## MicroRNA Expression Profiling with Recovered RNA

| | RNA Stabilization Replicate 1 | RNA Stabilization Replicate 2 |
|---|---|---|
| **% False Negative** | 0.63% | 0.94% |
| **% False Positive** | 3.02% | 3.85% |
| **% Concordance to Frozen Control** | 96.35% | 95.10% |

Expression profiling using the Agilent miRNA microarray. Total RNA samples (20 µg) containing miRNA were stored in the dry state for 2 weeks at 25°C with RNA Stabilization and compared with a control stored at -80°C. A strong concordance was observed between the RNA Stabilization samples and the frozen control.

## Long-Term RNA Stabilization at Ambient Temperatures




After a 6 month incubation period at 25ºC, 37ºC, and 56ºC, RNA samples treated with RNA Stabilization were stored at ambient temperature (25ºC) for 3 years. Recovered RNA was suitable for RT-PCR of a ~300 bp 18S fragment as shown in the 2% agarose gel. RNA quality was also evaluated on the Agilent Bioanalyzer, producing an average RIN score of 8.1.

## Frequently Asked Questions (FAQ)

1. **How do I use RNA treated with RNA Stabilization?**

   There are two options.

   Option 1: Following application to RNA Stabilization tubes, use RNA in a dissolved state for up to 100 hours at room temperature (21-25°C) or on ice. RNA Stabilization tubes convey stability to dissolved RNA by inactivating trace RNase, simplifying sample handling. After 100 hours, dry the sample down, or store at –80°C.

   Option 2: Following application to RNA Stabilization tubes, dry RNA and store or transport at ambient temperature. Following rehydration, RNA Stabilization conveys additional stability to dissolved RNA for up to 8 hours at room temperature (21-25°C) or on ice. Following the 8-hour post-recovery period, dry your sample down again for storage or store at –80°C.

2. **Can RNA be rehydrated and dried multiple times?**

   Yes, samples treated with RNA Stabilization can be rehydrated and re-dried up to five times.

3. **Is it safe to keep RNA at room temperature (21-25°C) during the 16-hour drying process?**

   Yes, the RNA Stabilization protects dissolved RNA at room temperature during the drying process.

## FAQ cont'd

4. **What is the composition of the storage solution after recovery?**

   After addition of molecular biology grade water, your samples will be in the same buffer as the original sample.

5. **How should I store my recovered RNA?**

   Following recovery, RNA may be stored for up to 8 hours at room temperature (21-25°C), and then dried down or stored at -80°C.

6. **Can I use the recovered RNA directly for downstream applications?**

   Yes, additional purification is not required prior to performing downstream applications. Similar RNA quality is maintained before and after recovery.

7. **What is contained within RNA Stabilization tubes? Is it composed of a filter, beads or paper?**

   RNA Stabilization tubes do not contain filters, beads, silica column, or paper. They contain a water soluble, chemical matrix.

## FAQ cont'd

**8. When removing DNA contamination from my RNA preparation, does the RNA Stabilization matrix interfere with DNase I digestion of gDNA?**

Yes, the RNA Stabilization matrix will inhibit DNase I activity. However, inhibition can be overcome by increasing the amount of DNase I used.

**9. Can RNA stabilization tubes be used to store DNA?**

No, the chemical matrix is specifically formulated to protect RNA, not DNA.

**10. Can I apply an input RNA volume that is less than the recommended 20 µL?**

Yes, but special care and handling is recommended. When applying the sample, be especially careful to place it in the very bottom of the tube. When recovering the sample we recommend using a minimum of twice the original volume to ensure you rehydrate all the sample.

**11. How can I avoid RNase contamination during the drying step?**

RNA Stabilization tubes contain a strong RNase inhibitor and is therefore protected during the drying period. However, if you are concerned about contamination, you can use Breathe Easier™ membranes for protection, but they do slow drying and extend the drying time. Using vacuum to speed the drying time may be required when using a barrier.

## FAQ cont'd

**12. Does the use of RNA Stabilization interfere with library construction?**

No, we have done many library preparations on samples treated with RNA Stabilization with no observed problems. Furthermore, as the sample moves through the library construction the chemical matrix is quickly diluted away.

**13. Will RNA Stabilization interfere with the removal of RNA template after first-strand cDNA synthesis?**

Yes, unless the RNA Stabilization matrix is diluted by a factor of >10-100 it will inhibit the RNase used to remove the starting RNA.

**14. Is there a minimum concentration of RNA that should be used?**

No, RNA Stabilization will protect even one molecule of RNA but volumes less than 10 µL and very small amounts of RNA are difficult to handle and recover.

**15. Will the RNA Stabilization tubes protect small RNA, miRNA, or tracrRNA?**

Yes, RNA Stabilization tubes will protect RNA molecules of any size, including small mRNA molecules. Because RNA Stabilization is a chemical matrix, not a binding matrix, the size of the RNA molecule does not affect its protection in the tubes.

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