

GenTegra RNAssure

GTR50-LQ

User Guide

**Immediate protection for purified RNA
eluting from the purification column**

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For Research Use Only

 GenTegra LLC

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Overview

GenTegra RNAssure incorporates GenTegra's Active Chemical Protection™ technology to stabilize RNA for at least 3 days at room temperature. RNAssure protects RNA during normal experimental handling where the sample temperature can often rise above 0 °C. Even brief exposure to elevated temperature is detrimental to RNA integrity, especially with prevalent contamination from endogenous or environmental nucleases. Samples stabilized with RNAssure can be used directly in downstream applications without further purification. RNAssure does not inhibit NGS library preparation, RT-PCR, or other expression profiling techniques. GenTegra RNAssure is available in standard 1.5 mL elution tubes (**Part No. GTR50-LQ**), as well as, 96-well plates (**Part No. GTR96-LQ**) ideal for automation. (For more information on various formats of GenTegra RNAssure, contact sales@gentegra.com)

Product Specifications

- GenTegra RNAssure is available in 1.5 mL elution tubes and in 96-well semi-skirted PCR plates
- Stabilizes RNA for up to 3 days at room temperature, 15-25°C
- Compatible with purified RNA from cell lines, whole blood, fresh and frozen tissues, and FFPE tissues
- Compatible with RNA purified using standard protocols and kits from all major suppliers (e.g., Invitrogen, Zymo, and QIAGEN)
- Compatible with all common storage buffers, including nuclease-free water, TE, and Tris buffers
- Recover in a volume of 20 – 150 µL of elution buffer

RNAssure Quick Reference Workflow

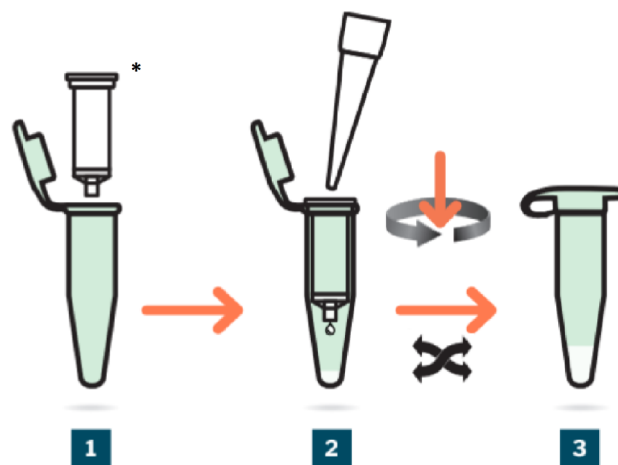
Follow standard protocol provided by RNA extraction kit manufacturer. Prior to the elution step use the RNAssure Elution Tube to replace the kit manufacturer's collection/elution/recovery tube.

Step 1: For the final elution step, insert the column into GenTegra RNAssure elution tube.

Step 2: Add between 20 – 150 µL elution buffer to the spin column and centrifuge to elute RNA. Briefly mix the eluted RNA by tapping the tubes or vortexing.

Step 3: Purified RNA is ready to be used at room temperature or on ice. RNAssure can stabilize RNA at room temperature for at least 3 days. See table for complete storage options.

STORAGE CONDITION	LENGTH OF PROTECTION
Room temperature (15 – 25 °C)	3 days
Refrigeration (2 – 8 °C)	2 weeks
Frozen (< -70 °C)	> 1 year



* Spin column supplied by RNA purification kit

Note: GenTegra RNAssure is designed to stabilize RNA in the liquid state by inactivating trace nucleases of all classes, protecting against oxidation, as well as helping prevent nonspecific adherence of RNA to plastic materials. RNA stabilized in GenTegra RNAssure can be used directly in downstream applications including quantitation, gel or Bioanalyzer™ analysis, RT-PCR, and NGS library preparation. No additional cleanup steps are needed.



Using RNAssure during DNase Treatment

Column-based RNA extraction from tissue samples often leads to genomic DNA (gDNA) contamination, and DNase digestion is routinely used to remove DNA contamination. During DNase treatment, RNA is exposed to RT or 37°C for extended periods of time, which may lead to significant RNA degradation. RNAssure protects RNA during DNase treatment while not interfering with the digestion of DNA.

Performing post-elution in-solution DNase digestion

1. If RNA was directly eluted to RNAssure tubes, perform DNase digestion according to kit manufacturers' standard protocols. The RNA sample is already protected by RNAssure, and no further precaution is required.
2. If RNA was not directly eluted to RNAssure tubes, transfer appropriate amount of RNA (20-150µl) to an RNAssure tube, mix thoroughly by vortexing or tapping, then perform DNase digestion according to kit manufacturers' standard protocols.

Performing on-column DNase digestion

1. Prepare DNase digestion working solution in an empty RNAssure tubes. Up to 300µl of DNase solution can be prepared in each RNAssure tube. If more than 300µl of DNase solution is need, prepare in multiple RNAssure tubes

Note: Preparing more than 300µl of DNase working solution per tube will lead to excessive dilution of the RNAssure stabilizer and may compromise its effectiveness

2. Follow kit manufacturers' standard protocols to perform on-column DNase digestion.

Product Specification RNAssure	Product Claims
Format	1.5 mL elution tubes, green color 96-well semi-skirted PCR plates
Shelf life	3 years (prior to use)
Total RNA application amount	≤ 30 µg
Sample Elution Volume	20 – 150 µL of elution buffer
Sample Stability	3 days at room temperature, 15- 25°C 2 weeks at 4°C Decades at -20C or dried
Buffer Compatibility	All common storage buffers, including nuclease-free water, TE, and Tris buffers
RNA Purification Kit Compatibility	Kits from all major suppliers
Drying	Samples may be dried at room temperature.
Recovery from dried	>99%
Stability when dry	Decades



GenTegra RNAssure Protocol

Drying and Storage of GenTegra RNAssure

1. Dry RNAssure solution according to the methods described in the table below.
 - Drying times will vary depending on application volume.
 - Whatever the drying method, ensure that RNA sample is completely dry prior to storage or shipping.
 - Use SpeedVac on room temperature setting (no additional heat or cooling).
 - Drying times for biosafety hood are approximate.

When using 1.5ml flip-top tubes in a FastDryer, volume must be $\leq 150 \mu\text{L}$.

2. When drying is complete, cap or seal tubes/plates and store at room temperature (21-25°C).

Volume	GenTegra FastDryer	Vacuum Desiccator or SpeedVac	Biosafety Hood
$\leq 50 \mu\text{L}$	16 hours	1-4 hours	24 hours
$\leq 100 \mu\text{L}$	32 hours	4-8 hours	48 hours
$\leq 150 \mu\text{L}$	48 hours	8-10 hours	64 hours

Shipping RNAssure protected sample for RNAseq

Maximum protection during shipment is provided by drying the RNA sample before shipping at ambient temperature.

1. Aliquot 50uL or half the total, whichever is less, to a new clean screw cap tube
 - GenTegra recommends a screw cap tube for more secure shipping. Flip top tube may be used but are more prone to the cap popping off during shipping
2. Dry both aliquots using a SpeedVac or Biosafety hood.
3. Store one aliquot at room temperature as your backup sample.
4. Ship the other aliquot at ambient temperature . No ice packs or dry ice are needed. Rehydrate the sample with the same volume of water as the aliquot.

Reconstituting and Using Dried RNAssure samples

1. Add a volume of molecular biology grade water equal to the original sample volume dried in the tube.
 - Mix gently using several pipette pumps.
2. Use no more than 2/3 of the total sample volume for subsequent analysis to endure no downstream interferences. Most analysis will use less than this.



Frequently Asked Questions

Q What is GenTegra RNAssure? Is GenTegra RNAssure composed of a filter, beads, or paper?

A GenTegra RNAssure is a water soluble, chemical matrix provided dried in a green microcentrifuge flip-top tube or 96-well semi-skirted PCR plate. RNAssure is not composed of any filters, beads, or paper.

Q Can I use the RNA in GenTegra RNAssure directly for downstream applications?

A Yes, additional purification is not required prior to performing downstream applications. The GenTegra RNAssure does not interfere with any downstream protocol.

Q When removing DNA contamination from my RNA preparation does the inhibitor in GenTegra RNAssure interfere with digestion of gDNA using DNase I?

A No, the inhibitor concentration used in RNAssure will not interfere with common DNase treatment protocol.

Q Can I elute an RNA sample in a volume that is smaller than the recommended 20 µL?

A Collecting less than 20 µL will increase the concentration of the active ingredient which may inhibit downstream analysis.

Q Can I use RNAssure with TRIzol or non-standard RNA purification kits?

A Yes, RNAssure Elution Tube can be used without the need for manufacturer kits with spin columns. After the final elution or resuspension step, collect 20 – 150 µL of the RNA sample and add it to the GenTegra RNAssure Elution tube. Mix by gently tapping for 10-15 seconds or pipetting up and down 10 times to solubilize and mix the RNAssure stabilization agent into the purified RNA sample.

Q Can I use RNAssure with whole blood stabilization tubes (PAXgene, Tempus, etc.)?

A Yes, RNAssure Elution Tubes are compatible with whole blood stabilization tubes.

Q Does the use of GenTegra RNAssure interfere with library construction?

A No, at its normal concentration there is no effect on the reverse transcriptase or polymerase reactions.

Q Will GenTegra RNAssure interfere with the removal of RNA template after the first strand reaction?

A RNAssure contains potent inhibitors of all RNases. To ensure proper removal of RNA templates, ensure that the input RNAssure stabilized RNA sample constitutes less than 10% of the total reaction volume.

Q Is there a minimum or maximum concentration of RNA that can be used?

A There is no limitation of RNA concentration. However, follow the RNA extraction kit manufacturer's recommendation and do not exceed the recommended total RNA amount.

Q Will GenTegra RNAssure work with small RNA molecules (e.g., miRNAs, tracrRNA, etc.)?

A Yes, GenTegra RNAssure is a chemical matrix that effectively protects all forms of RNA.

Q Will the presence of GenTegra RNAssure change my 260/280 or 260/230 ratio?

A GenTegra RNAssure will not affect 260/280 ratio. However, it has an absorbance of approximately 0.4 OD at 230 nm (e.g. NanoDrop analysis) and will cause a slightly higher 230 nm sample reading and therefore reduce the 260/230 ratio value. If desired, a "blank" or control sample can be made. To do this, add same volume of nuclease-free water to an empty RNAssure tube.

GenTegra

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