GenTegra®-RNA dry Bulk User Guide

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GenTegra

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FOR RESEARCH USE ONLY

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Simplified Workflow

Apply RNA to GenTegra-RNA tubes/wells

↓

Dry for storage or shipping at ambient temperature

↓

To recover, reconstitute with molecular biology grade water

↓

RNA is ready for use

Overview

GenTegra®-RNA is a novel technology for storage and transport of RNA in ready to use aliquots. GenTegra-RNA allows storage of RNA in a water-free environment, which protects samples from hydrolysis, oxidation and microbial growth. Simply add purified RNA, dry, and store at room temperature. When needed, simply rehydrate and the RNA sample is ready for downstream analysis. GenTegra-RNA is well suited for ambient temperature shipping locally, nationally and internationally, tolerating the rigorous United States Military ambient shipping specifications of -80°F (-62°C) to 160°F (71°C). In addition to standard GenTegra tubes and microplates, GenTegra-RNA is available in bulk form for custom applications:

- 1x concentration – For making custom tubes for stabilizing and storing purified RNA samples.
- 5x concentration – For adding GenTegra-RNA directly to liquid samples of purified RNA, followed by drying.

Product Information

	GenTegra-RNA Bulk
Catalog Number	GTR100-B
Product form	Dried material in 2 ml vial
Sample Volume	20-45 µl
Sample Amount	0.05-25 µg of RNA
RNA concentration	Any
Recovery Volume	Equivalent to Sample volume
1X for 100 custom tubes	Add 1.65 ml molecular biology grade water
Amount per tube	15 µl per tube
5X for addition to 100 samples	Add 0.55 ml molecular biology grade water
Amount per sample	Add 5 µl to each sample
Drying Method	SpeedVac, Vacuum Desiccator, FastDryer™, Biosafety Hood

Upon arrival, GenTegra-RNA is a dry white material in the bottom of the vial. In the dry form GenTegra-RNA has a shelf life estimated to be at least three years. When re-hydrated the solution should be used within 2 weeks.

GenTegra RNA 1X Protocols

The 1X concentration solution can be applied to tubes or microplate wells. After air drying, it forms a GenTegra matrix at the bottom, which can then be stored dry until needed. Purified RNA in solution can then be added to these matrix-coated tubes or plates, to be followed by air-drying and storage.

Preparing 1X GenTegra RNA Tubes and Microplates

1. Add 1.65 ml of water to the GenTegra-RNA bulk tube and dissolve with gentle intermittent vortexing for 1-5 minute.
2. Aliquot 15 µl of GenTegra-RNA solution into your tubes.
3. Dry tubes by any of these methods:
   - In a SpeedVac at ambient temperature or 30°C till dry, ~2-3 hours
   - In an vacuum desiccator for ~3-4 hours
   - In an GenTegra FastDryer overnight ~16 hours
   - In a Biosafety Hood overnight ~16 hours

If the tubes do not dry overnight, then additional effort should be taken to reduce humidity and drying time to less than 24 hours. Do not use heat to accelerate the drying process.

Applying RNA Samples to Stabilized Storage Containers

1. Aliquot RNA samples into the prepared GenTegra tubes or microplate. The typical containers used are 0.3 ml, 1.7 ml, and 0.5 ml tubes, and 96-well microplates.
2. Mix by pipetting up and down 6 times to solubilize the GenTegra-RNA Matrix.
3. Proceed to the protocol "Drying and Storing GenTegra" on page 8. After the samples are dry, they are stable for long term ambient storage.

GenTegra-RNA 5X Protocol – Adding to Samples

The 5X concentration solution is added directly to liquid purified RNA samples. After mixing, the solution can be dried and is stable for long term storage.

1. Add 0.55 ml of water to the GenTegra-RNA BULK tube and dissolve using gentle mixing.
2. Add 5 µl of GenTegra-RNA 5x to each isolated RNA sample.
   The RNA sample that may be used:
   - Volume: 20-45 µl
   - Amount: 0.05-25 µg
2. Mix thoroughly and gently to disperse the GenTegra Matrix and avoid foaming
3. Quickly centrifuge to bring the matrix and sample to the tube/well bottom.
4. Dry by any of these methods:
   - In a SpeedVac at ambient temperature
   - In a GenTegra FastDryer overnight or till dry
   - In a vacuum desiccator till dry.
   - In a Biosafety Hood overnight or till dry

   If the tubes do not dry overnight, then additional effort should be taken to reduce humidity and drying time to less than 24 hours. Do not use heat to accelerate the drying process. After the samples are dry, they are completely stable for long term ambient storage.

Using Liquid RNA Stabilized with GenTegra-RNA

GenTegra-RNA is designed to stabilize RNA in the liquid state by inactivating trace nucleases.

RNA stabilized in GenTegra-RNA may be used either at room temperature (21-25°C), or on ice.

1. Apply GenTegra-RNA to RNA according to the protocol on page 6 or add RNA sample to tubes containing dry GenTegra-RNA.
2. Use liquid RNA stabilized in GenTegra for RNA aliquots destined for prompt use (i.e. for quantitation, gel/Bioanalyzer analysis or any downstream application).
    - RNA stored in GenTegra-RNA may be used for up to 100 hours in liquid form at room temperature (21-25°C), or on ice, with increased stability.
3. Following the 100 hour period, dry the sample down or store RNA according to your typical protocol.

Drying and Storing GenTegra-RNA

1. Dry tubes with caps off, according to either of the methods described in the table below.
    - The original caps may be saved and re-used when drying is complete. Alternately, new caps may be purchased from most laboratory supply companies.
    - Axygen microcentrifuge tube screw caps are compatible with GenTegra-RNA tubes and are available from Genesee Scientific in a variety of colors and styles.
    - Drying time for SpeedVac® is approximate.
    - Refer to page 19 for FastDryer operation instructions.
    - Refer to page 21 for instructions on drying RNA in a SpeedVac.
2. When drying is complete, cap tubes and store or transport GenTegra-RNA tubes at ambient temperature.

Application Volume	Drying Time		
	FastDryer	Vacuum Desiccator or SpeedVac	Biosafety hood
20 µL	16 hours	~2 hours	~18 hours
21-50 µL	16 hours	~4 hours	~24 hours

Drying RNA Using a GVGT2001 FastDryer

A FastDryer may be used to dry up to 50 µL of RNA solution.

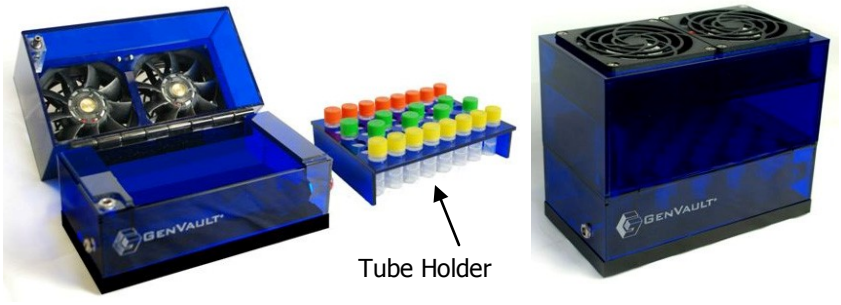
- Refer to FastDryer User Guide, www.GenTegra.com, for detailed instructions.
- A GVGT2001 FastDryer may also be used for drying one rack of 0.3 ml duster tubes. Please refer to the FastDryer User Guide for details; www. GenTegra.com

1. Ensure that the FastDryer is plugged in.
2. Place unsealed or uncapped tubes or rack in tube/rack holder.
3. Close the FastDryer lid.
4. Turn on the FastDryer by pressing the red ON/OFF switch.

Blue lights will illuminate when FastDryer is operating.

5. Dry overnight (16 hours).
6. Remove samples and cap or seal for storage/transport.

For details on operation and use of the FastDryer refer to the GenTegra FastDryer User Guide.



FastDryer GVGT2001

Drying RNA using a SpeedVac

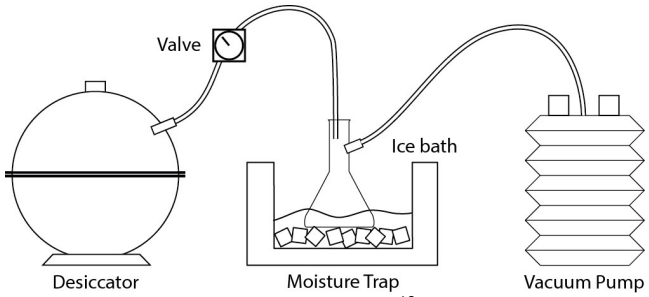
A SpeedVac may be used to dry up to 50µL of RNA. Drying times are approximate and may need to be modified based on the specifications of your SpeedVac. On the first use, ensure that tubes are completely dry by visually inspecting or attempting to pipette liquid from the bottom of the tube.

1. Place tubes with lids off in the SpeedVac.
2. Ensure that the temperature setting does not exceed 30°C.
3. Dry tubes for approximately 2-4 hours, according to guidelines in the table below.
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 50µL of RNA. Drying time is approximate and may need to be modified based on the system. The system consists of a vacuum desiccator, vacuum pump, a vapor trap, assorted tubing and a small ice bath. After the first use, ensure that tubes are completely dry by visually inspecting or attempting to pipette liquid from the bottom of the tube.

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



RNA Sample Recovery

1. Add a volume of molecular-grade water **equivalent to the input volume**.
2. Incubate the tubes at **room temperature** (21-25°C) for 10 minutes.

   Do not attempt to recover RNA on ice.

3. Pipette up and down 10 times to solubilize the RNA.
4. Alternately, tubes may be capped, vortexed for 10 seconds and centrifuged briefly.
5. The RNA is ready for use in downstream applications.
6. RNA recovered from GenTegra-RNA may be used for up to **8 hours in liquid form** at room temperature (21-25°C), or on ice, with increased stability.
7. Following the 8 hour period post recovery, dry down or store recovered RNA according to your typical protocol.

Multiple Drying and Rehydration of RNA

Following recovery, an aliquot of RNA may be removed for use, and the sample dried again. This procedure may be repeated multiple times until a maximum of 75% of the original sample (and thus, GenTegra chemical matrix) is removed.

For example, a 50 µl sample is applied to a GenTegra tube, dried and rehydrated. Following rehydration, 10 µl is removed for analysis, leaving 40 µl (80% of the original sample), which is dried again. This process can be repeated until removal of an aliquot for analysis causes the volume of the sample to drop below 20 µl (or <25% of the original sample), in which case it should be stored according to typical conditions (for example, at -20 °C). These calculations assume that the sample was always rehydrated at the same concentration.

This calculation is based on percentage of matrix remaining in the solution and not absolute volume.

Technical Information

Expected Results

- Quantitative recovery of RNA
  - Quality is comparable to input RNA

Storage and Transport

- Quantitative recovery of RNA
- Quality is comparable to input RNA

Transport conditions: -80°C to +56°C

Storage conditions: 15°C to 30°C

Tested Storage Buffers Compatible with GenTegra-RNA

- Qiagen Buffer AE
- TE, pH 7.5 and TE pH 8.0 (10mM Tris and 1mM EDTA)
- Low EDTA TE, pH 8.0 (10mM Tris and 0.1mM EDTA)

Tested Applications Compatible with GenTegra-RNA

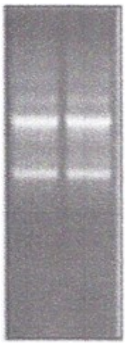
The following applications have been tested to be compatible with RNA recovered from GenTegra-RNA tubes:

- Gene Expression Analysis
- Genotyping
- Sequencing
- HLA Typing

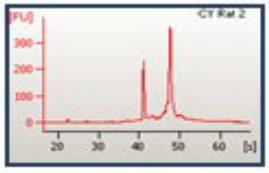
Storage of Rat Liver RNA in GenTegra-RNA

Storage of Rat Liver RNA in GenTegra-RNA

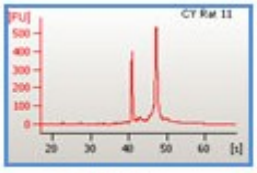
GenTegra Protected 25°C | Frozen Control –80°C



GenTegra Protected 25°C | Frozen Control –80°C



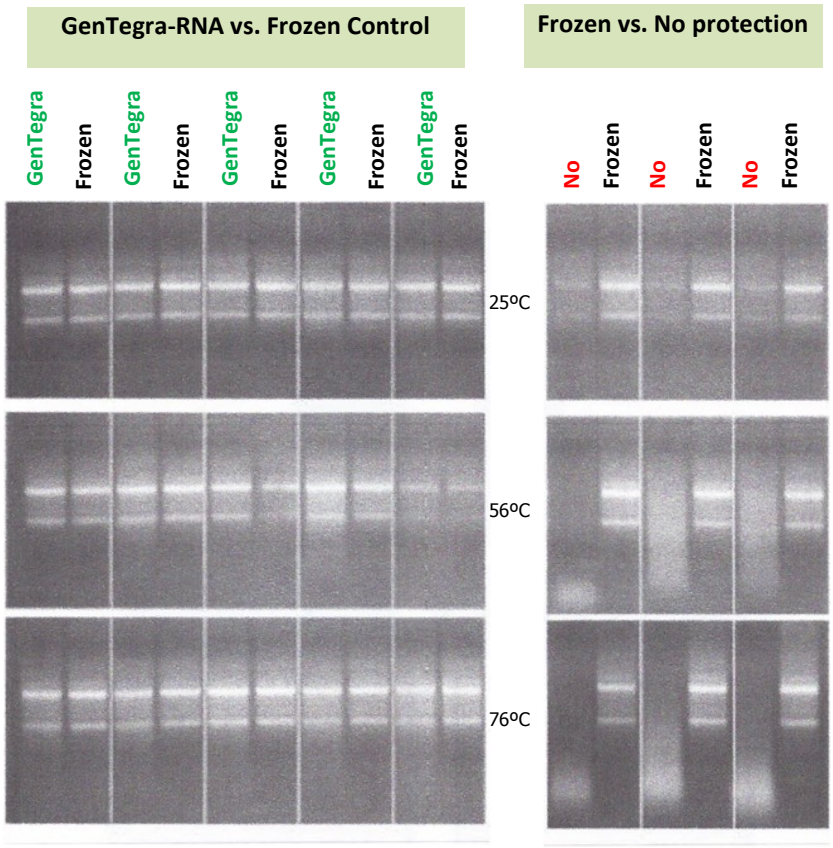
RIN 10



RIN 10

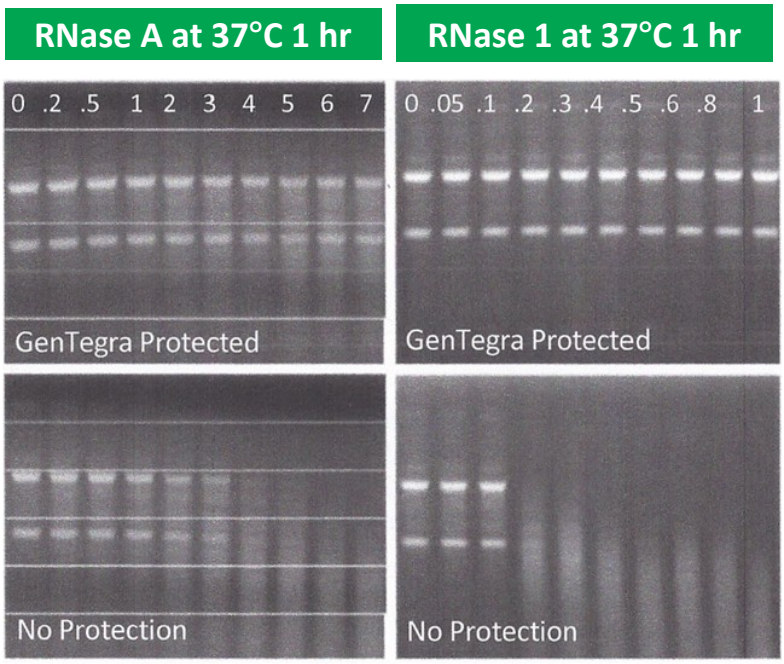
Quality and integrity of RNA stored in the presence of GenTegra -RNA is identical to RNA stored frozen. Total RNA (20µg) purified from rat liver was stored in the dry state at 25°C for 30 days and compared with a control stored frozen at -80°C. RNA integrity was examined by running on a 0.8% agarose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

Storage of PAXgene RNA in GenTegra-RNA



The integrity of PAXgene RNA stored in the presence of GenTegra-RNA is equivalent to RNA stored frozen. RNA was purified from individual PAXgene tubes, and split into two aliquots. One aliquot of each sample was stored frozen at -80°C, while the other was stored in the dry state for 30 days at 25°C, 56°C or 76°C in the presence or absence of GenTegra-RNA.

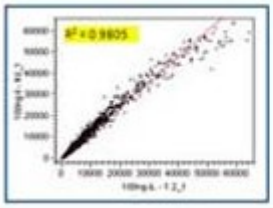
GenTegra-RNA Increases RNA stability in the liquid State in the Presence of Trace RNase



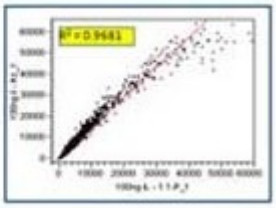
RNA integrity is maintained in the liquid state in the presence of increasing amounts of RNase A and RNase 1 only when protected with GenTegra-RNA. HeLa cell RNA (5 µg) was incubated with the indicated amounts of RNase (unit is $x10^9$ molecule) at 37ºC for one hour in the presence or absence of GenTegra-RNA.

Illumina Expression Profiling with RNA Recovered from GenTegra-RNA

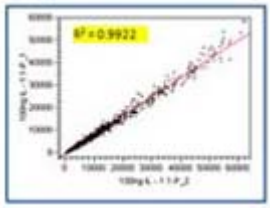
Replicate 1 Frozen vs. GenTegra



Replicate 2 Frozen vs. GenTegra

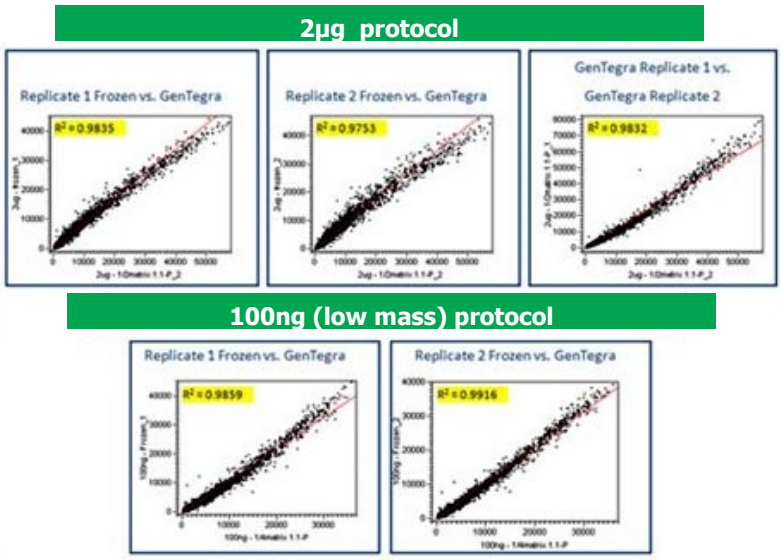


GenTegra Replicate 1 vs. GenTegra Replicate 2



Successful expression profiling of RNA using Illumina HT-12 Expression Beadchips. Replicate RNA samples purified from HeLa cells (20µg) were stored in the dry state for two weeks at 25°C with GenTegra-RNA and compared with a control stored at -80°C.

Affymetrix Expression Profiling with RNA Recovered from GenTegra-RNA



Successful 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 two weeks at 25°C with GenTegra-RNA and compared with a control stored at -80°C.

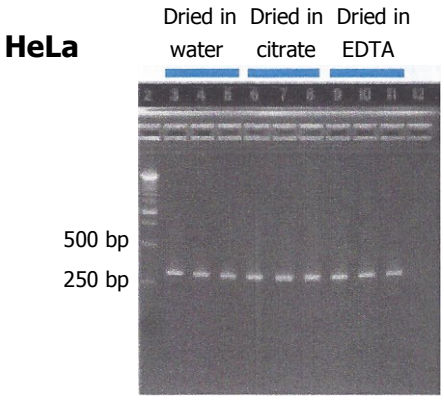
MicroRNA Expression Profiling with RNA Recovered from GenTegra-RNA

GenTegra-RNA Matrix			
	Replicate 1 (1/5x)	Replicate 2 (1/5x)	1/10/x
% False Negative	0.63%	0.94%	3.13%
% False Positive	3.02%	3.85%	2.08%
% Concordance to Frozen (960 probes interrogated, ~30% positive calls)	96.35%	95.10%	94.70%

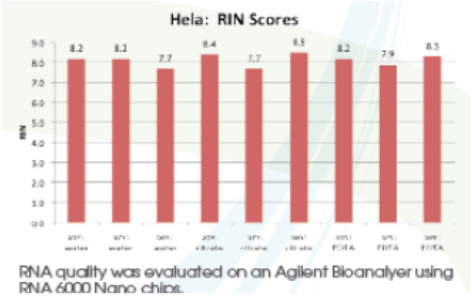
Successful expression profiling of RNA using the Agilent miRNA microarray. Total RNA samples (20µg) containing miRNA were stored in the dry state for two weeks at 25°C with GenTegra and compared with a control stored at - 80°C.

Ongoing ambient temperature experiments show retention of qualify for 3.5 years

After a six month incubation period at 25ºC, 37ºC, and 56ºC samples stored on GenTegra-RNA were kept at ambient temperature (25ºC) for three years duplicating actual storage conditions. The RNA quality was suitable for RT-PCR of a ~300 bp 18S fragment as shown in the 2% agarose gel. The samples correlate with the RIN scores.



2% agarose gel corresponding to the RIN scores shown in chart below.
Lanes 3, 6, 9 are 26ºC, lanes 4, 7, 10 are 37ºC and lanes 6, 8, 11 are 56ºC.



RNA quantity was evaluated on an Agilent Bioanalyzer using RNA 6000 Nano chips.

Frequently Asked Questions (FAQ)

What are the two options for stabilizing RNA in GenTegra -RNA?

Option 1 – Following application to GenTegra-RNA, use RNA in liquid form for up to 100 hrs on ice or at room temperature (21-25°C). GenTegra-RNA conveys additional stability to RNA in liquid form by inactivating trace RNase, simplifying sample handling. Following the 100 hour period, dry the sample down, or store RNA according to your typical protocol.

Option 2 - Following application to GenTegra-RNA dry RNA solution and store or transport at ambient temperature. Following rehydration, GenTegra-RNA conveys additional stability to RNA in liquid form 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 according to your typical protocol.

Where can I purchase new caps for GenTegra-RNA screw cap tubes? Are looped/tethered caps available?

New caps may be purchased from any lab supply company. Axygen microcentrifuge tube screw caps are compatible with GenTegra-RNA tubes and are available through several distributors: Genesee Scientific offers the caps in a variety of colors and styles, including looped/tethered caps, at: www.geneseesci.com

Can RNA be rehydrated and dried multiple times?

Yes, RNA on GenTegra-RNA can be rehydrated and re-dried up to five times.

Can I use the 5X bulk to make customer tubes?

We recommend using the 1X concentration so a larger surface area at the bottom of the tube is coated with GenTegra-RNA but it is just as acceptable to deposit 5 µl of 5X in the bottom of the tube.

Frequently Asked Questions (FAQ) cont'd

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

Yes, the GenTegra-RNA protects RNA in the liquid state at room temperature during the drying process.

What is the composition of the storage solution after recovery?

After addition of molecular-grade water, your samples will be in the same buffer they were stored in at the time of application.

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 dry down or store according to your typical protocol.

Can I use the recovered RNA directly for downstream applications?

Additional purification is **not** required prior to performing downstream applications. Similar RNA quality is maintained before and after recovery.

What is GenTegra-RNA? Is GenTegra-RNA composed of a filter, beads or paper?

GenTegra-RNA is not a filter, beads or paper. GenTegra-RNA is a water soluble, inert chemical matrix.

Can GenTegra-RNA tubes be used to store DNA?

No, the chemical matrix used to store DNA is not the same as the chemical matrix used to store RNA. Use GenTegra-DNA tubes for storage and transport of purified DNA samples.

Notes Page

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