FastGene™ RNA save
User Guide

Version A **October 2018**

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FOR RESEARCH USE ONLY

Product Specifications

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

Storage and Transport

- Store and transport at ambient temperature

Simplified Workflow

Apply RNA to FastGene™ RNA save tubes

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Dry for storage or shipping at ambient temperature

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To recover, reconstitute with molecular biology grade water

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RNA is ready for use

FastGene™ RNA save Protocol

RNA Application

1. Add ≤ 20 µg of RNA in a volume of 20-50 µl. To ensure complete mixing of RNA and the FastGene™ RNA save, apply a minimum volume of 20 µl. For concentrated samples, add water to a final volume of ≥20-50 µl
2. Incubate for 5 minutes at room temperature (21-25°C).
3. Mix by pipetting up and down 10 times to solubilize and mix in the GenTegra matrix. The FastGene™ RNA save is supplied as a coating at the bottom of each tube.
4. For continued use of RNA in liquid form, proceed to protocol on page 6.
5. For transport or long-term storage of RNA, proceed to drying protocol on page 7.

Using Liquid RNA Stabilized with FastGene™ RNA save

FastGene™ RNA save is designed to stabilize RNA in the liquid state by inactivating trace nucleases and protecting against oxidation.

RNA stabilized in FastGene™ RNA save may be used either at room temperature (~23°C), or on ice.

1. Apply RNA to FastGene™ RNA save according to the protocol on page 5.
2. Use liquid RNA stabilized in RNA save for RNA aliquots destined for prompt use (i.e. for quantitation, gel/Bioanalyzer analysis or any downstream application).
    - RNA stored in FastGene™ RNA save may be used for up to 100 hours in liquid form at room temperature (~23°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 FastGene™ RNA save Samples

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 GenTegra (catalog # GTR5201-S).
    - Axygen microcentrifuge tube screw caps are compatible with FastGene™ RNA save tubes.
    - Drying time for SpeedVac® is approximate.
    - Refer to page 8 for FastDryer operation instructions.
    - Refer to page 9 for instructions on drying RNA in a SpeedVac.
2. When drying is complete, cap tubes and store or transport FastGene™ RNA save tubes at ambient temperature.

Application Volume	Drying Time		
	FastDryer (P.8)	Vacuum Desiccator or SpeedVac	Biosafety Hood
20 µL	10 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.

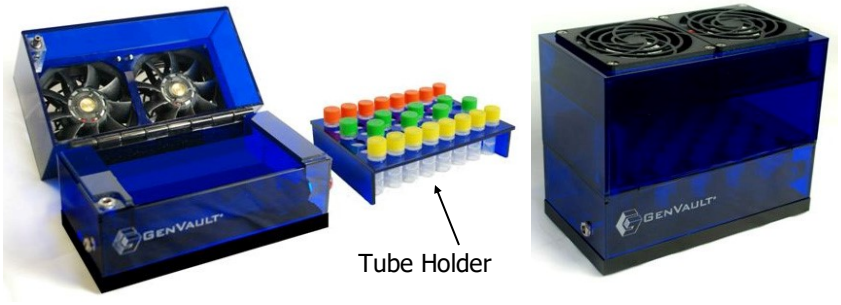
- Refer to page 20 for detailed instructions.
- A GVGT2001 FastDryer may also be used for drying one rack of 0.3ml duster tubes. Please refer to the FastDryer user manual for details at; www.gentegra.com

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

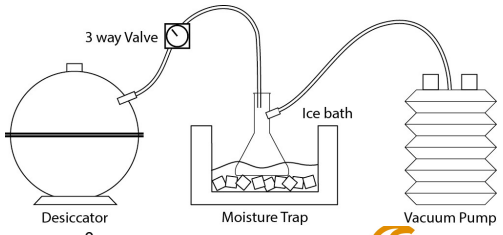
A SpeedVac may be used to dry up to 50 µL of RNA. Drying times are approximate and may need to be modified based on the specifications of your SpeedVac. On the first use, ensure that tubes are completely dry by visually inspecting or attempting to pipette liquid from the bottom of the tube.

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 guide-lines 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 50 µL 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 Recovery at Sequencing Laboratory

1. Add a volume of molecular-grade water **equivalent to the input volume**.
2. Incubate the tubes at room temperature (~23°C) for 10-minutes.

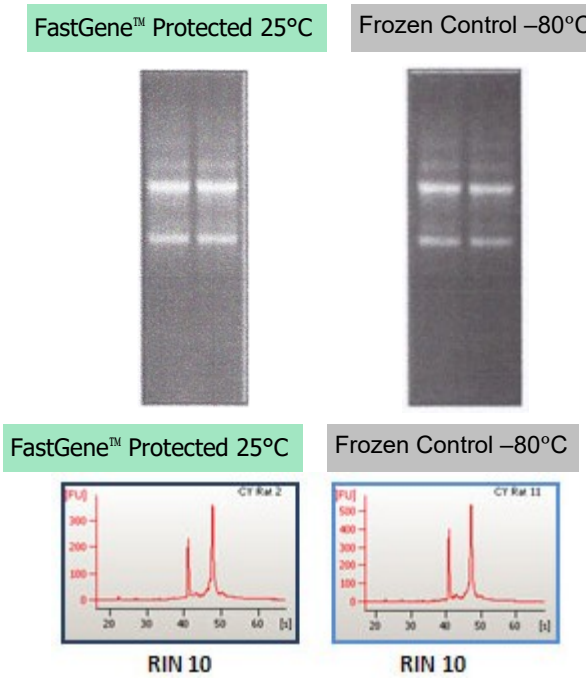
   Do not attempt to recover RNA on ice.
3. Pipette up and down 10 times to solubilize the RNA.
4. Alternately, tubes may be capped, vortexed for 10 seconds and centrifuged briefly.
5. The RNA is ready for use in QC or downstream applications.
6. RNA recovered from FastGene RNA Save may be used for up to **8 hours in liquid form** at room temperature (~23°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.

Product Information

FastGene™ RNA save 0.5mL Screw-cap microtubes	
Catalog #	FG-GTR05-3(Trial) FG-GTR05-100
Tube Volume	0.5 mL
Application Volume	20-50 µL
Application Volume	1-20 µl require special handling
Application Amount	≤ 20 µg
Concentration (RNA application)	Any
Recovery Volume	Equivalent to application volume
Concentration (RNA recovery)	Any
Drying Method	FastDryer or SpeedVac

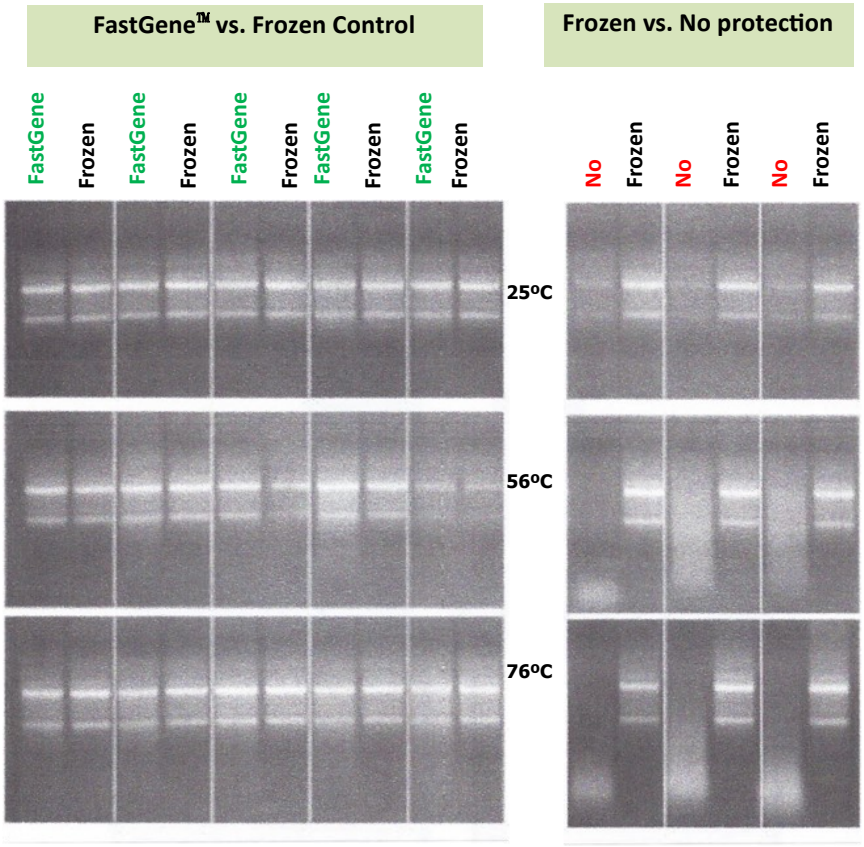
Technical Information

Storage of Rat Liver RNA in FastGene™ RNA save



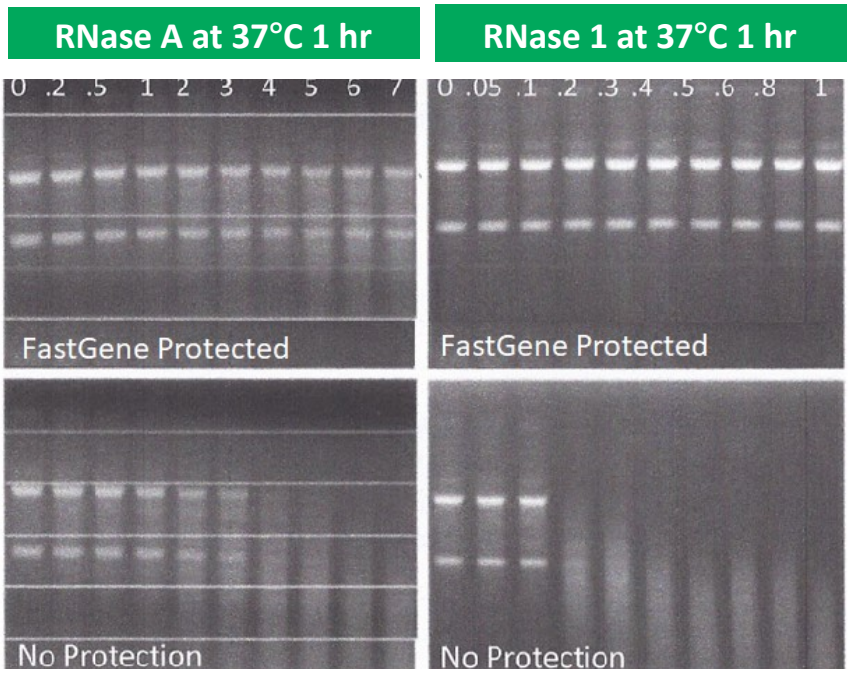
Quality and integrity of RNA stored in the presence of FastGene™ RNA save is identical to RNA stored frozen. Total RNA (20 µg) purified from rat liver was stored in the dry state at 25°C for 30 days and compared with a control stored frozen at -80°C. RNA integrity was examined by running on a 0.8 agarose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

Storage of PAXgene® RNA in FastGene™ RNA save



The integrity of PAXgene® RNA stored in the presence of FastGene™ RNA save 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℃, while the other was stored in the dry state for 30 days at 25℃, 56°C or 76°C in the presence or absence of FastGene™ RNA save.

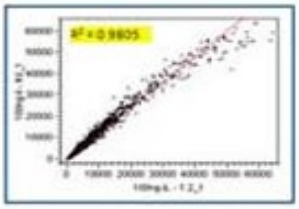
FastGene™ RNA save Increases RNA stability in the liquid State in the Presence of Trace RNase



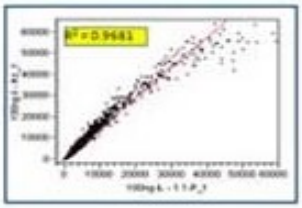
RNA integrity is maintained in the liquid state in the presence of increasing amounts of RNase A and RNase 1 only when protected with FastGene™ RNA save. HeLa cell RNA (5 µg) was incubated with the indicated amounts of RNase (unit is $x10^9$ molecule) at 37ºC for one hour in the presence or absence of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

Illumina Expression Profiling with RNA Recovered from FastGene™ RNA save

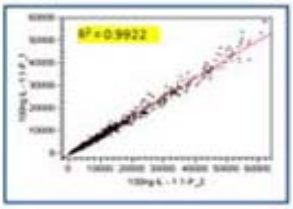
Replicate 1 Frozen vs. Fast-Gene™



Replicate 2 Frozen vs. Fast-Gene™

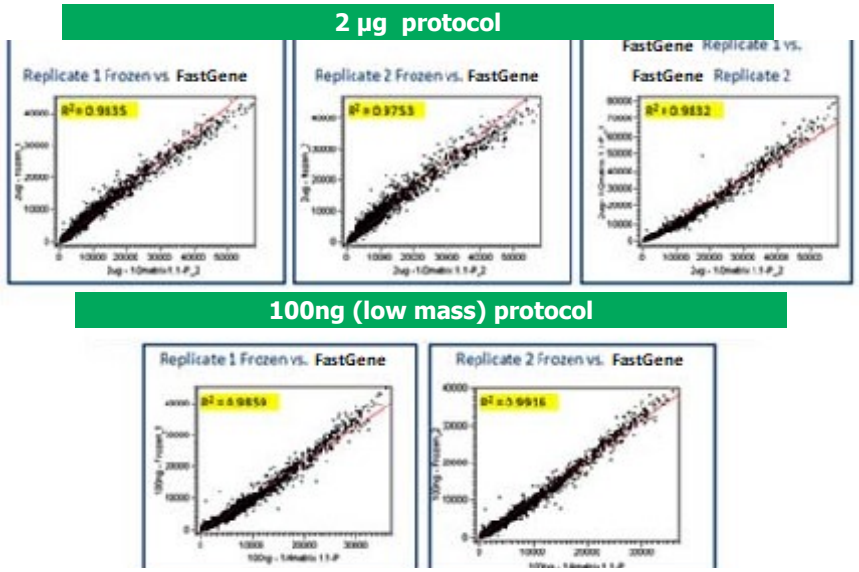


FastGene™ Replicate 1 vs. FastGene™ Replicate 2



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

Affymetrix Expression Profiling with RNA Recovered from FastGene™ RNA save



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 FastGene™ RNA save and compared with a control stored at -80°C.

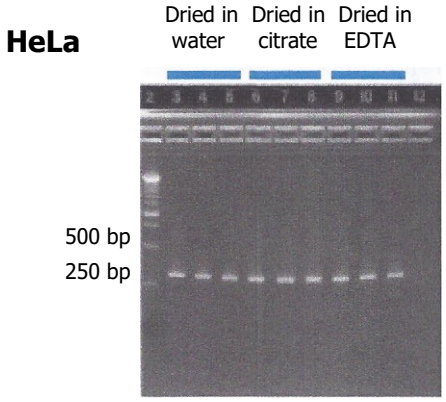
miRNA Expression Profiling with RNA Recovered from FastGene™ RNA save

FastGene™ RNA save 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%

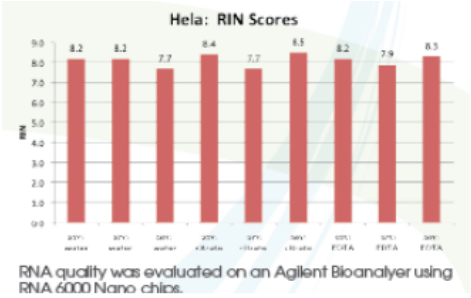
Successful expression profiling of RNA using the Agilent miRNA microarray. Total RNA samples (20µg) containing miRNA were stored in the dry state for two weeks at 25°C with FastGene™ RNA save and compared with a control stored at - 80°C.

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 FastGene™ RNA save 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 37 ºC 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 FastGene™ RNA save?

Option 1 – Following application to FastGene™ RNA save, use RNA in liquid form for up to 100 hrs at room temperature (21-25°C) or on ice. FastGene™ RNA save 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 FastGene™ RNA save dry RNA and store or transport at ambient temperature. Following rehydration, FastGene™ RNA save 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 FastGene™ RNA save screw cap tubes? Are looped caps available?

New caps may be purchased from GenTegra. Axygen microcentrifuge tube screw caps are also compatible with FastGene™ RNA save tubes.

(Customer support : info@genetics-n.co.jp)

Can RNA be rehydrated and dried multiple times?

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

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

Yes, the FastGene™ RNA save protects RNA in the liquid state at room temperature during the drying process.

Frequently Asked Questions (FAQ) cont'd

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 FastGene™ RNA save? Is FastGene™ RNA save composed of a filter, beads or paper?

FastGene™ RNA save is not a filter, beads, silica column or paper. FastGene™ RNA save is a water soluble, chemical matrix.

When removing DNA contamination from my RNA preparation does the inhibitor in FastGene™ RNA save interfere with digestion of gDNA using DNase I.?

Yes, the RNase inhibitor in FastGene™ RNA save is an inhibitor of DNase I. However, because of the low concentration of inhibitor it should be easy to swamp the inhibition by simply increasing the amount of DNase I used.

Can FastGene™ RNA save 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 FastGene™ DNA save tubes for storage and transport of purified DNA samples.

(Customer support : info@genetics-n.co.jp)

Frequently Asked Questions (FAQ) cont'd

Can I apply an RNA sample that is smaller 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. Refer to our tutorial on handling small sample volumes for further details.

How can I avoid RNase contamination during the drying step? Is there a special protecting membrane available?

FastGene™ RNA save has a strong inhibitor for RNases and is therefore protected against any possible contamination by RNases during its drying period. However, if you are concerned about this 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 such as the Breath Easier membrane.

Does the use of FastGene™ RNA save interfere with library construction?

At its normal concentration range there is no effect on the polymerase reaction and we have done many library preps on FastGene™ RNA save protected samples with no problems at all. Furthermore, as the sample moves thought the library construction the FastGene™ RNA save is quickly diluted away.

Will FastGene™ RNA save interfere with the removal of RNA template after the first strand reaction?

Yes, unless the FastGene™ RNA save is diluted by >10-100 times compared to its normal concentration it will be a potent inhibitor of the RNase used to remove the starting RNA.

Frequently Asked Questions (FAQ) cont'd

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

No, GenTegra-DNA will protect even one molecule of RNA but volume less than 10 µL and very small amounts of RNA are difficult to handle and easy to lose on side walls of tubes and pipette tips.

Will the FastGene™ RNA save work with small RNA, e.g. miRNAs or tracrRNA (67nt)? Is there a minimum length for the RNA to bind efficiently to your columns? ?

Yes, FastGene™ RNA save is a chemical matrix which will protect small RNAs as well as it protects long mRNAs. Because FastGene™ RNA save is a chemical matrix it does not change how it interacts with and protects short or long RNAs.

Notes

1061 Serpentine Lane, Suite B — Pleasanton, CA 94566