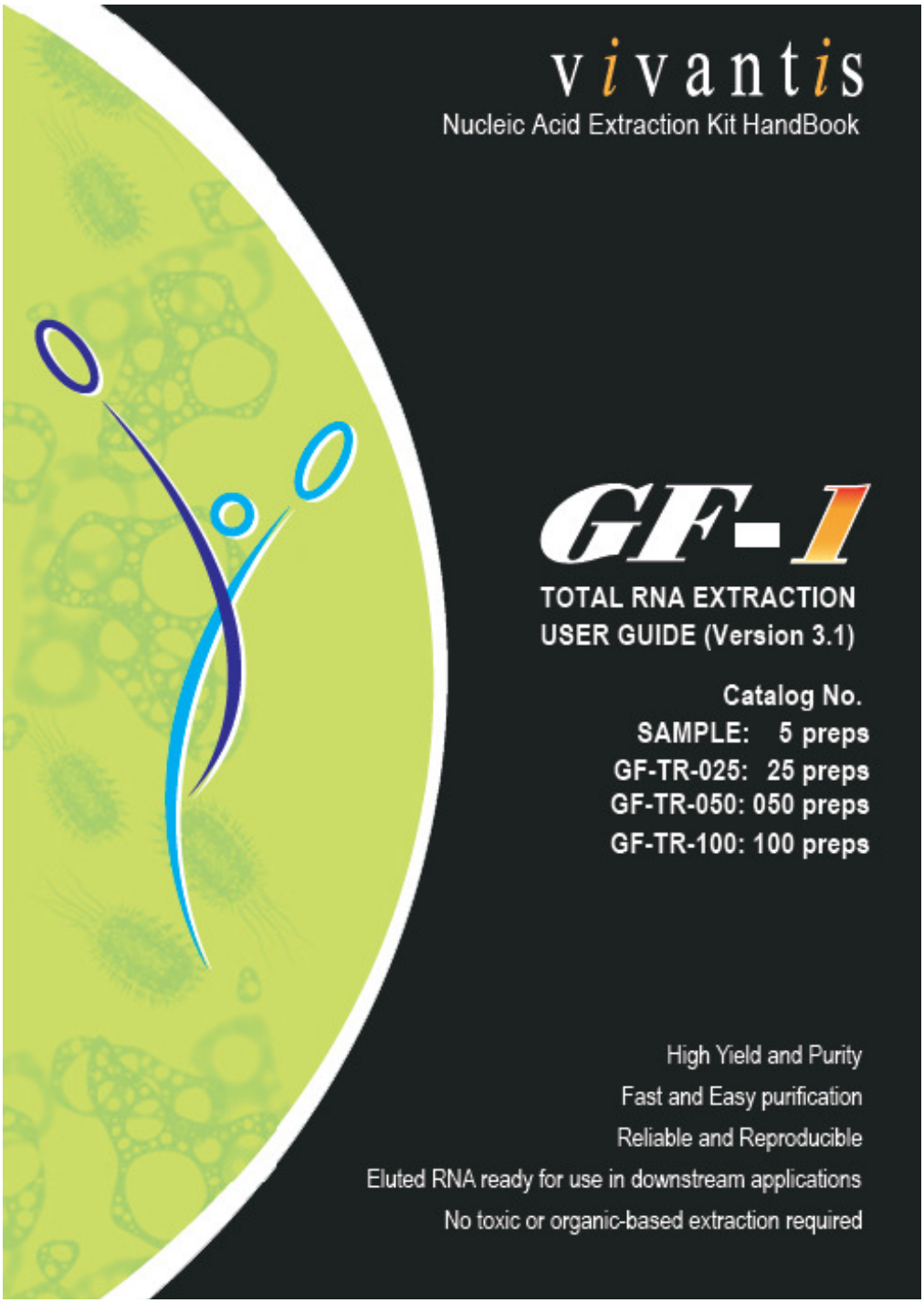

vivantis
Nucleic Acid Extraction Kit HandBook
GF-1
TOTAL RNA EXTRACTION
USER GUIDE (Version 3.1)
Catalog No.
SAMPLE: 5 preps
GF-TR-025: 25 preps
GF-TR-050: 050 preps
GF-TR-100: 100 preps
High Yield and Purity
Fast and Easy purification
Reliable and Reproducible
Eluted RNA ready for use in downstream applications
No toxic or organic-based extraction required

Introduction

The **GF-1 Total RNA Extraction Kit** is designed for the isolation of total RNA (longer than 200 bases) from a variety of sources such as animal tissues, bacteria cells and cell cultures. Samples are lysed in the presence of a specially formulated buffer which inactivates cellular RNases. Meanwhile, fragments of DNA are simply removed by applying the sample into a specially designed homogenization column followed by DNase I treatment. Optimized buffer and ethanol are added to provide selective binding of RNA onto the column matrix while contaminants are efficiently washed away. High-quality RNA is then eluted in RNase-free water. Isolated RNA is ready for use in downstream applications such as dephosphorylation, kinasing, blotting, cDNA synthesis, etc.

Kit components

Product Catalog No.	5 Preps SAMPLE	25 Preps GF-TR-025	50 Preps GF-TR-050	100Preps GF-TR-100
Components				
RNA binding columns	5	25	50	100
Homogenization columns	5	25	50	100
Collection tubes	10	50	100	200
Buffer TR*	4ml	20ml	40ml	80ml
Inhibitor Removal Buffer*	1.5ml	8ml	16ml	30ml
Wash Buffer*	3ml	15ml	30ml	2 X 30ml
DNase I*	0.04ml	0.2ml	0.4ml	0.8ml
Digestion Buffer	0.35ml	2ml	4ml	8ml
Digestion Enhancer	0.04ml	0.2ml	0.4ml	0.8ml
RNase-free Water	1ml	5ml	10ml	20ml
Proteinase K*	0.06ml	0.3ml	0.6ml	1.1ml
Handbook	1	1	1	1

* Please refer to **Reconstitution of Solutions** and **Storage and Stability.**

The **GF-1 Total RNA Extraction Kit** is available as 25, 50 and 100 purifications per kit.

The reagents and materials provided with the kit are for research purposes only.

Additional Materials to be Supplied by User

80% Ethanol
Absolute Ethanol (>95%)
2-mercaptoethanol
Lysozyme (Bacterial RNA Purification)
Lyticase/ Zymolase (Yeast RNA Purification)

Reconstitution of Solutions

The bottle labeled **Inhibitor Removal Buffer** and **Wash Buffer** contain concentrated buffer which must be diluted with absolute ethanol (>95%) before use.

For **SAMPLE (5 preps)**,

Add **1.5ml** of absolute ethanol into the bottle labeled **Inhibitor Removal Buffer**.
Add **7ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

For **GF-TR-025 (25 preps)**,

Add **8ml** of absolute ethanol into the bottle labeled **Inhibitor Removal Buffer**.
Add **35ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

For **GF-TR-050 (50 preps)**,

Add 16**ml** of absolute ethanol into the bottle labeled **Inhibitor Removal Buffer**.
Add 70**ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

For **GF-TR-100 (100 preps)**,

Add 30**ml** of absolute ethanol into the bottle labeled **Inhibitor Removal Buffer**.
Add 70**ml** of absolute ethanol into the bottle labeled **Wash Buffer**.
Add 70**ml** of absolute ethanol into the other bottle labeled **Wash Buffer** only prior to use.

Store **Inhibitor Removal Buffer** and **Wash Buffer** at room temperature with bottle capped tight after use.
DNase I is sensitive to physical denaturation. **DO NOT VORTEX**. Mix gently by inverting the tube.
Prepare **DNase I** in 7µl aliquots to avoid repeated freeze-thaw cycles. Store at -20°C.

Storage and Stability

Store all solutions **(EXCEPT DNase I)** at 20°C-30°C.
Store **Proteinase K** at -20°C.
Store **DNase I** at -20°C.
Buffer TR is stable for 1 month after addition of 2-mercaptoethanol (prepare as needed).
Kit components are guaranteed to be stable for 18 months from the date of manufacture. **Buffer TR** and **Inhibitor Removal Buffer** may exhibit salt precipitation due to cold temperature. If this occurs, simply warm the bottle at 55°C - 65°C with occasional mixing until completely dissolved.

Chemical Hazard

Buffer TR and **Inhibitor Removal Buffer** contain guanidine salts which can be harmful when in contact with skin or swallowed. Always wear gloves and practice standard safety precautions. Do NOT disinfect guanidine or extraction waste in solutions containing bleach or any form of acid. To clean any items contaminated with the reagent, simply soak in detergent and water to remove all traces of guanidine before cleaning with bleach or acidic solutions.

Procedures

- All steps are to be carried out at room temperature unless otherwise stated.
- Do not exceed the maximum recommended starting volume of sample to prevent reduction in yield and purity.
- Be certain not to introduce any RNases during the whole purification process. Wear gloves at all times.
- Add 10µl of 2-mercaptoethanol into 1ml of **Buffer TR** before use. **Buffer TR** is stable for 1 month upon addition of 2-mercaptoethanol.
- For each purification, prepare the **DNase I Digestion Mix** as follows (prepare as needed):

DNase I	**7µl**
Digestion Buffer	**56 µl**
Digestion Enhancer	**7 µl**

Mix the **DNase I Digestion Mix** by gentle pipetting **(DO NOT VORTEX)**.
Store the **DNase I Digestion Mix** on ice while performing the initial steps of RNA extraction.

I. Sample preparation

A. Animal Tissue (Except Skeletal Tissue)

Note 1: Do not use more than 30mg tissues.
Note 2: Frozen animal tissue should not be allowed to thaw during handling.
Note 3: It is more difficult to extract total RNA from skeletal tissue such as heart and muscle tissue with this standard protocol. Therefore, users are advised to use the protocol as described in Animal Tissue II to extract total RNA from heart and muscle tissue.

1. Grind animal tissue (maximum: 30mg) in liquid nitrogen to fine powder using a pre-chilled mortar and pestle. Place the ground material into a pre-chilled, RNase-free vessel of suitable size for homogenization at the next step.
Ensure that the tissue is ground into fine powder to obtain a high yield of RNA. Frozen tissue should not be allowed to thaw during handling.

2. Homogenize the tissue completely in 700µl **Buffer TR** by using a conventional rotorstator or any other suitable homogenizer.
*Ensure that 2-mercaptoethanol is added into **Buffer TR** before use.*

3. Centrifuge the sample at maximum speed for 3 min.

4. Transfer the lysate into a **Homogenization column** assembled in a collection tube. Centrifuge at maximum speed for 2 min. Save the flow-through.

5. Add 650µl of 80% ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
Precipitate may form after the addition of ethanol.

6. Proceed to **ll. RNA Isolation.**

B. Animal Tissue II (Skeletal Tissue)

Note 1: Do not use more than 30mg tissues.

Note 2: Frozen animal tissue should not be allowed to thaw during handling.

1. Grind animal tissue (maximum: 30mg) in liquid nitrogen to fine powder using a pre-chilled mortar and pestle. Place the ground material into a pre-chilled, RNase-free vessel of suitable size for homogenization at the next step.

Ensure that the tissue is ground into fine powder to obtain a high yield of RNA. Frozen tissue should not be allowed to thaw during handling.

2. Homogenize the tissue completely in 300µl **Buffer TR** by using a conventional rotor-stator or any other suitable homogenizer.

*Ensure that 2-mercaptoethanol is added into **Buffer TR** before use.*

3. Add 590µl water to the lysate, followed by addition of 10µl **Proteinase K** solution. Mix thoroughly by pulse vortexing. Incubate at 65°C for 10min.

4. Centrifuge the sample at maximum speed for 3 min.

5. Transfer the lysate (max.650µl) into a **Homogenization column** placed in a collection tube. Centrifuge at maximum speed for 2 min. Save the flow-through. Repeat for the remaining sample from step 4.

6. Add 450µl of absolute ethanol to the flow-through. Mix thoroughly by pipetting.

7. Proceed to **II. RNA Isolation**.

C. Bacteria Cell

Note 1: Do not use more than $1x10^9$ bacteria as this will overload the column.

Note 2: Prepare lysozyme of the recommended concentration in TE buffer pH 8.0.

For Gram-negative bacteria, prepare 0.5mg/ml.

For Gram-positive bacteria, prepare 5mg/ml.

1. Pellet bacteria from culture grown to log phase or early stationary phase by centrifugation at 6,000 x *g* for 5 min at 4°C. Discard the supernatant completely.

Do not use more than $1x10^9$ bacteria.

2. Resuspend the bacteria pellet in 100µl of
   a) 0.5mg/ml lysozyme for Gram-negative bacteria strains. Mix by vortexing and incubate at room temperature for 3 - 5 min.
   b) 5mg/ml lysozyme for Gram-positive bacteria strains. Mix by vortexing and incubate at room temperature for 5 - 10 min.
   Ensure that the lysozyme used is prepared in TE buffer.

3. Add 350µl **Buffer TR** to the sample.
*Ensure that 2-mercaptoethanol is added into **Buffer TR** before use.*

4. Transfer the lysate into a **Homogenization column** assembled in a collection tube. Centrifuge at maximum speed for 2 min. Save the flow-through.

5. Add 300µl absolute ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
Precipitate may form after the addition of ethanol.

6. Proceed to **II. RNA Isolation**.

D. Cell Culture

Note 1: Do not use more than $1x10^7$ cells as it will overload the column.

1. Pellet cell culture in a microcentrifuge tube at 1,000 x *g* for 5 min.
Do not use more than $1x10^7$ cells.

2. Add 350µl **Buffer TR** to the cell pellet. Mix thoroughly by vortexing vigorously.
*Ensure that 2-mercaptoethanol is added into **Buffer TR** before use.*
*Volume of **Buffer TR** is added according to different number of cells,*
*If number of cells is $<5x10^6$, add 350µl **Buffer TR.***
*If number of cells is $5x10^6$ - $1x10^7$, add 700 µl **Buffer TR**.*

3. Transfer the lysate into a **Homogenization column** assembled in a collection tube. Centrifuge at maximum speed for 2 min. Save the flow-through.

4. Add equal volume of 80% ethanol (350µl or 700µl) to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
Precipitate may form after the addition of ethanol.

5. Proceed to **II. RNA Isolation**.

E. Plant Tissue

Note 1: Do not use more than 100mg tissues.
Note 2: Frozen tissue should not be allowed to thaw during handling.
Note 3: Certain plant tissues with high metabolites may solidify in Buffer TR; therefore, users are advised to use Buffer HR (Cat. no.: GFHR025), which is sold separately.

1. Grind plant tissue (maximum: 100mg) in liquid nitrogen to fine powder using a pre-chilled mortar and pestle. Place the ground material into a cold, RNase-free 2ml microcentrifuge tube.
Ensure that the tissue is ground into fine powder to obtain a high yield of RNA.
Frozen tissue should not be allowed to thaw during handling.

2. Add 400µl **Buffer TR** or **Buffer HR** (refer to note 3) to the ground tissue. Mix thoroughly by vortexing vigorously.
*Ensure that 2-mercaptoethanol is added into **Buffer TR** before use.*

3. Centrifuge sample at maximum speed for 3 min.

4. Transfer the lysate into a **Homogenization column** assembled in a collection tube. Centrifuge at maximum speed for 2 min. Save the flow-through.

5. Add 350µl of 80% ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
Precipitation may form after the addition of ethanol.

6. Proceed to **II. RNA Isolation**.

F. Yeast

Note 1: Use only freshly harvested cells.
Note 2: Do not use more than 5 x 10^7 cells as this will overload the column.
Note 3: Prepare Buffer YL (1M Sorbitol, 0.1M EDTA) fresh for every purification by adding in 0.1% 2-mercaptoethanol and lyticase / zymolase (50U per 1 x 10^7 cells) before use.

1. Pellet yeast cells by centrifugation at 5,000 x *g* for 5 min at 4°C. Discard the supernatant completely.
Do not use more than 5 x10^7 yeast cells.

2. Resuspend the cells in 1.5ml Buffer YL containing lyticase / zymolase and 0.1% 2-mercaptoethanol (refer note 3). Incubate at 30°C for 30 min to generate spheroplasts.
Use only freshly harvested cells.

3. Pellet the resulting spheroplasts by centrifugation at 1,000 x *g* for 5 min

.

4. Add 350µl **Buffer TR** to lyse spheroplasts. Mix thoroughly by vortexing vigorously.
*Ensure that 2-mercaptoethanol is added to **Buffer TR** before use.*

5. Transfer the lysate into the **Homogenization column** placed in a collection tube. Centrifuge at maximum speed for 2 min. Save the flow-through.

6. Add 350µl of 80% ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
Precipitate may form after addition of ethanol.

7. Proceed to **II. RNA Isolation**.

II. RNA Isolation

1. Transfer the sample (max. 650µl) including any precipitate into a **RNA Binding Column** assembled in a collection tube. Centrifuge at 10,000 x *g* for 1 min. Discard the flow-through.

2. Add 500µl of **Wash Buffer** and centrifuge at maximum speed for 1 min. Discard flow through.

3. Pipette 70µl of **DNase I Digestion Mix** into **RNA Binding Column** and incubate at room temperature for 15 min. Ensure the digestion mix is pipette directly onto the membrane.
Some samples may have high genomic DNA content. Prolong the incubation time (need to be determined empirically) if necessary.

4. Add 500µl of **Inhibitor Removal Buffer** and centrifuge at maximum speed for 1 min. Discard flow through.

5. Add 500µl of **Wash Buffer** and centrifuge at 10,000 x *g* for 1 min. Discard flow-through.

6. Repeat wash with 500µl of **Wash Buffer** and centrifuge at 10,000 x *g* for 1 min. Discard flow-through.

7. Centrifuge the column at 10,000 x *g* for 1 min to remove traces of buffer.

8. Place the column into a new microcentrifuge tube. Add 40-60µl of **RNase-free Water** directly onto the membrane and stand for 1 min. Centrifuge at 10,000 x *g* for 1 min. Store RNA at -20°C or -80°C.

Troubleshooting

Please note that by not adhering to the recommended protocols, unsatisfactory results related to yield and quality of the RNA may occur. If problems arise, please refer to the following:

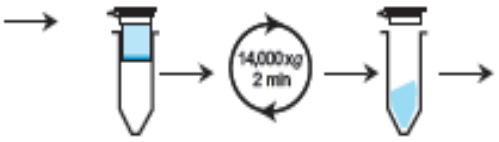
Problem	Possibility	Suggestions
Homogenization column clogged	*Insufficient sample disruption or homogenization*	*Tissue sample need to be ground till become fine powder by using mortar pestle.*
		Homogenize tissue sample with rotor-stator homogenizer or pass the homogenate through a 18-21 gauge needle several times till visible tissue fragment is eliminated.
	Too much starting materials	*Reduce amount of starting material in the subsequent purification.*
	Lysate is too viscous	*Users may dilute homogenate with additional lysis buffer.*
Low RNA yield	*Absolute ethanol or 80% ethanol is not added prior loading lysate onto RNA binding column*	*Repeat purification with new sample.*
	80% ethanol is prepared wrongly	*Repeat purification with new sample.*
	Inhibitor Removal Buffer *and* **Wash Buffer** *are reconstituted wrong*	*Please refer to "Reconstitution of Solutions". Repeat purification with new sample.*
RNA degradation/ smearing	*Samples not properly stored*	*Tissue sample should be flash-frozen immediately in liquid nitrogen prior storing at -70 °C.*

Problem	Possibility	Suggestions
	Inappropriate handling	*Frozen tissue sample should not be thawed during handling.* *Use disposable plasticware and plastic tips.* *Ensure that the purification is performed in an RNase-free environment.* *Wear gloves at all times.*
Genomic DNA contamination	*Absolute ethanol or 80% ethanol is not added prior loading onto RNA binding Column*	*Repeat purification with new sample.*
	80% ethanol is prepared wrongly	*Repeat purification with new sample.*
	Lysate is not passed through **Homogenization Column** *prior loading onto* **RNA Binding Column**	*Repeat purification with new sample.*
	DNase I *treatment is not performed properly*	*Please refer to preparation of* **DNase I Digestion Mix** *and pipette the* **DNase I Digestion Mix** *directly onto the membrane.*
Poor performance of eluted RNA in downstream Applications	*Eluted RNA contains traces of ethanol*	*Ensure that the column is spun dried prior to elution.*
	RNA degraded	*Please refer to problem "RNA degradation/smearing".*

I Sample Preparation

I) Lyse and homogenize sample by using **Buffer TR** (Cultured cells, plant and animal tissues)

II) Enzymatic lysis with addtion of **Buffer TR**
Bacteria cells (Lysozyme)
Yeast cells (Lyticase or Zymolase)
Skeletal tissues (**Proteinase K**)



Loading to Homogenization Column

Addition of Absolute Ethanol or 80% Ethanol

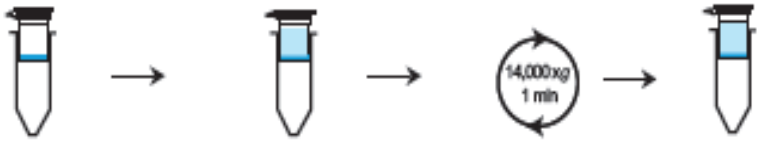
II RNA Isolation

Loading to RNA Binding Column

Centrifuge
Discard flow through

Column Washing
Add **Wash Buffer**

Centrifuge
Discard flow through

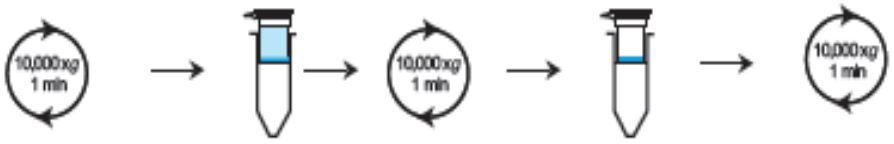


DNase Treatment
Incubate at RT, 15 min

Column Washing
Add **Inhibitor Removal Buffer**

Centrifuge
Discard flow through

Column Washing
Add **Wash Buffer**



Centrifuge
Discard flow through

Column Washing
Add **Wash Buffer**

Column Drying

Elution
Transfer column to a new microcentrifuge tube.
Add 40-60µl
RNase-free water.
Stand for 1 min

Centrifuge
Store RNA at -20ºC or -70ºC

Contact : info@vivantechnologies.com