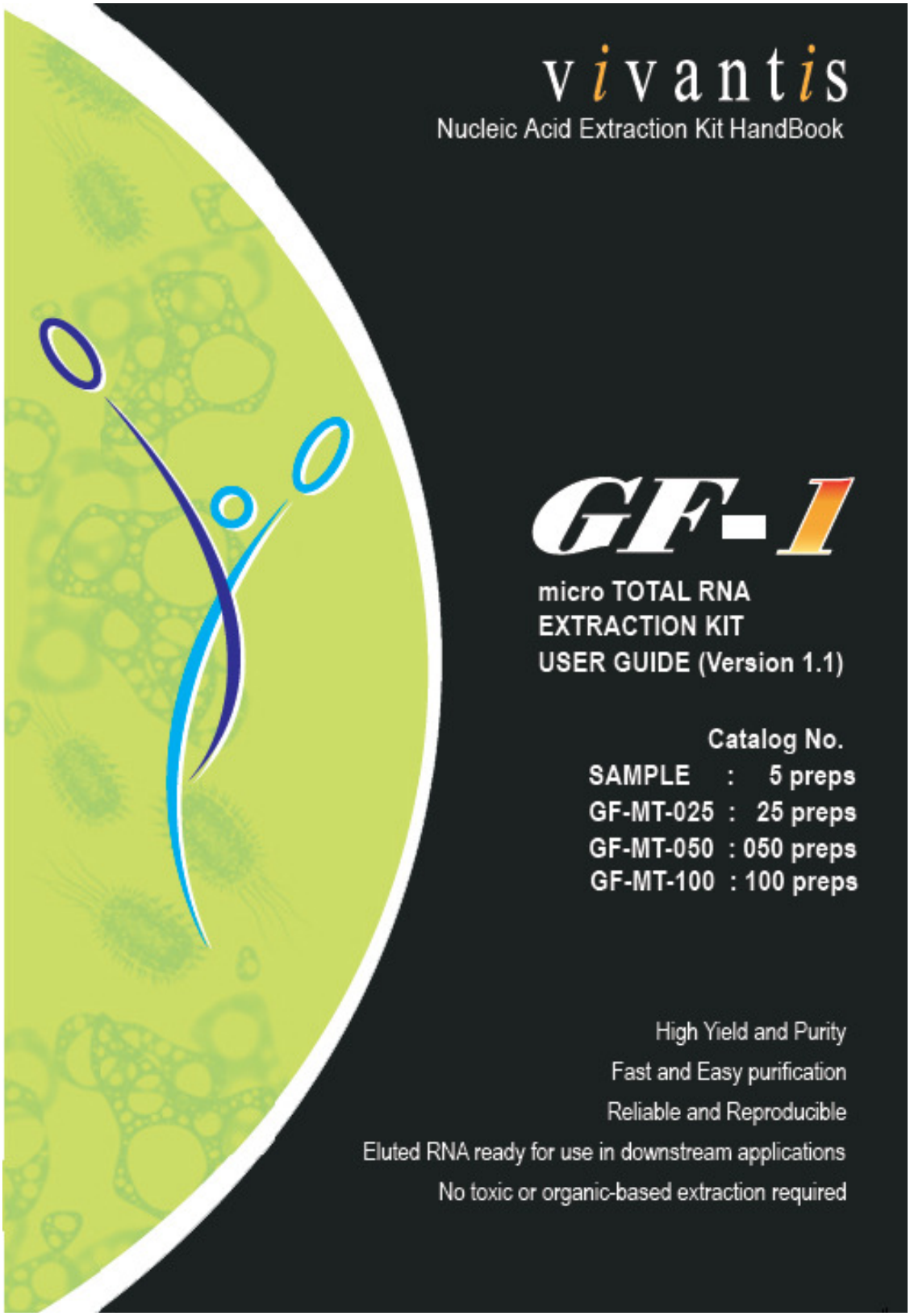
vivantis

Nucleic Acid Extraction Kit HandBook



GF-1

micro TOTAL RNA EXTRACTION KIT
USER GUIDE (Version 1.1)

Catalog No.

SAMPLE : 5 preps
GF-MT-025 : 25 preps
GF-MT-050 : 050 preps
GF-MT-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 microTotal RNA Extraction Kit** is designed for the isolation of total RNA (large RNAs, siRNAs, microRNAs, and viral RNAs) from a variety of sources such as animal tissues, blood, serunm, plasma, and cell cultures. Samples are lysed in the presence of a specially formulated buffer which inactivates cellular RNases. Optimized buffer is 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, transcription profiling, kinasing, blotting, cDNA synthesis, etc.

Kit components

Product Catalog No.	25 Preps GF-MT-025	50 Preps GF-MT-050	100Preps GF-MT-100
Components			
RNA binding columns	25	50	100
Collection tubes	25	50	100
Buffer TS*	13ml	26ml	52ml
Wash Buffer*	6ml	12ml	24ml
RNase-free Water	5ml	10ml	20ml
Handbook	1	1	1

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

The **GF-1 microTotal 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

Absolute Ethanol (>95%)
Isopropanol (>98%)
PBS

Reconstitution of Solutions

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

For **GF-MT-005-S (5 preps)**,

Add **3.4ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

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

Add **24ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

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

Add **34ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

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

Add **34ml** of absolute ethanol into the bottle labeled **Wash Buffer**.
Add **34ml** of absolute ethanol into the other bottle labeled **Wash Buffer** only prior to use.

Store **Wash Buffer** at room temperature with bottle capped tight after use.

Storage and Stability

- Store all solutions at 20°C-30°C.
- Kit components are guaranteed to be stable for 18 months from the date of manufacture. **Buffer TS** 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 TS contains 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.

I. Sample preparation

A. Animal 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 700µl **Buffer TR** by using a conventional rotorstator or any other suitable homogenizer (20-G needle, Polytron, etc).

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

4. Transfer the lysate into a clean 1.5ml microcentrifuge tube.

5. Proceed to **ll. RNA Isolation.**

B. Monolayer Cell Culture

Note 3: Recommended to use 1 x 10^4 – 5 x 10^6 cells per prep.

1. Add 100µl **Buffer TS** per cm^2 dish/plate (refer to the table below) directly on the surface area of the adherent monolayer cell culture. Mix thoroughly by pipetting.

Approximate confluent cell number per culture area.

Culture Container	Well/Flask Surface Area	Cell Number
6-well plate	9-10 cm^2	0.5-1x10^6
12-well plate	4 cm^2	4-5x10^5
24-well plate	2 cm^2	1-3x10^5
96-well plate	0.3-0.6 cm^2	4-5x10^4
T25 Culture Flask	25 cm^2	2-3x10^6
T75 Culture Flask	75 cm^2	0.5-1x10^7
T175 Culture Flask	175 cm^2	2-3x10^7

Example: Add 400µl of Buffer TS per well of a 12-well plate.

2. Transfer the mixture into a clean 1.5ml microcentrifuge tube. Vortex vigorously to lyse the cells.

3. Proceed to **II. RNA Isolation**.

C. Suspension Cells

Note 4: Recommended to use 1 x 10^4 – 5 x 10^6 cells per prep.

1. Centrifuge cells (up to 5 x 10^6 cells) at 10,000 x *g* for 2 min. Remove the supernatant carefully without disturb the pellet.

2. Resuspend the cells by adding 100µl (one volume) of PBS and 300µl (3 volume) of **Buffer TS**. Vortex to lyse the cells.

3. Centrifuge at 10,000 x *g* for 2 min to remove particulates.

4. Transfer the mixture into a clean 1.5ml microcentrifuge tube.

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

D. Biological Fluids

Note 5: 100µl of sample (whole blood, plasma, serum, buffy coat, CSF, semen, saliva, biological fluids containing RNA virus and body fluids) per prep.

1. Add 300µl (3 volume) of **Buffer TS** into 100µl of biological fluid. Mix well by vortexing.

2. Centrifuge at 10,000 x *g* for 2 min to remove particulates.

3. Transfer the mixture into a clean 1.5ml microcentrifuge tube.
When transfer supernatant of whole blood or plasma, avoid red blood phase at the bottom of the tube.

4. Proceed to **II. RNA Isolation**.

II. RNA Isolation

1. Add one volume of isopropanol into the sample homogenate and mix well by vortexing.
Example: Add 300µl of isopropanol into 300µl of sample lysate.

2. Transfer the sample into a **RNA Binding Column** assembled in a collection tube. Centrifuge at 10,000 x *g* for 1 min. Discard the flow-through.

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

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

5. Centrifuge the column at max speed for 3 min to remove traces of buffer.

6. 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	*Isopropanol is not added priorloading lysate onto RNAbinding column*	*Repeat purification with new sample.*
	Wash Buffer *are reconstituted wrong*	*Please refer to "Reconstitution of Solutions". Repeat purification with new sample.*
	Samples not properly stored	*Tissue sample should be flash-frozen immediately in liquid nitrogen prior storing at -70°C.*

Problem	Possibility	Suggestions
RNA degradation/ smearing	*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.*
	Isopropanol is not added priorloading onto RNA binding olumn	*Repeat purification with new sample.*
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

Lyse and homogenize sample by using Buffer TS (animal tussues, cultures cells, and biological fluids.

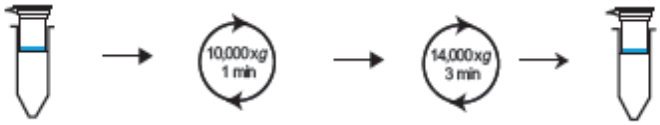
II RNA Isolation

Loading to RNA Binding Column

Centrifuge
Discard flow through

Column Washing
Add **Wash Buffer**

Centrifuge
Discard flow through



Column Washing
Add **Wash Buffer**

Centrifuge
Discard flow through

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

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