



vivantis
Nucleic Acid Extraction Kit Handbook

GF-1

BLOOD TOTAL RNA
EXTRACTION KIT USER GUIDE

Catalog No.
SAMPLE: 5 Preps
GF-TB-025: 25 preps

High Yield and Purity
Fast and Easy purification
Reliable and Reproducible
Eluted DNA ready for use in downstream applications
No toxic or organic-based extraction required

Introduction

The **GF-1 Blood Total RNA Extraction Kit** is designed for the isolation of total RNA (longer than 200 bases) from up to 1ml fresh or frozen anti-coagulated whole blood. 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, blotting, cDNA synthesis, etc.

Kit components

Product Catalog No.	5 Preps SAMPLE	25 Preps GF-TB-025
Components		
Homogenization Columns	5	25
RNA Binding Columns	5	25
Collection tubes	10	50
Buffer BR*	2ml	10ml
Inhibitor Removal Buffer*	1.5ml	8ml
Wash Buffer*	3ml	15ml
Proteinase K*	0.11ml	0.55ml
DNase I*	0.04ml	0.2ml
Digestion Buffer	0.35ml	2ml
Digestion Enhancer	0.04ml	0.2ml
RNase-free Water	1.ml	10ml
Handbook	1	1

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

The **GF-1 Blood Total RNA Extraction Kit** is available as 25 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%)

β -mercaptoethanol

Reconstitution of Solutions

The bottles 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**.

DNase I is sensitive to physical denaturation. **DO NOT VORTEX**. Mix gently by inverting the tube. Prepare **DNase I** in 7ml aliquots to avoid repeated freeze-thaw cycles.

Store at -20°C.

For **GF-TB-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**.

DNase I is sensitive to physical denaturation. **DO NOT VORTEX**. Mix gently by inverting the tube. Prepare **DNase I** in 7ml aliquots to avoid repeated freeze-thaw cycles.

Store at -20°C.

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

Storage and Stability

- Store all solutions (EXCEPT **DNase I** and **Proteinase K**) at 20°C-30°C.
- Store **DNase I** and **Proteinase K** at -20°C.
- **Buffer BR** is stable for 1 month after addition of β -mercaptoethanol (prepare as needed). Kit components are guaranteed to be stable for 12 months from the date of manufacture. **Buffer BR** 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 BR 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

Reminders

- All steps are to be carried out at room temperature unless otherwise stated.
- Do not exceed 1ml of blood 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.
- Pre-set waterbath to 65°C.
- Add 10µl of β-mercaptoethanol into 1ml of **Buffer BR** before use. **Buffer BR** is stable for 1 month upon addition of β-mercaptoethanol.
- For each purification, prepare the **DNase I Digestion Mix** as follows (prepare as needed):

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

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.

1. **Blood lysis**

Add 400µl of blood sample into a clean microcentrifuge tube. Add 300µl of **Buffer BR** to the blood sample and mix thoroughly by pulse-vortexing. Add 180µl of **RNase-free Water**. Add 20µl of **Proteinase K** and mix immediately. Incubate at 65°C for 10 min.

Ensure that β-mercaptoethanol is added into Buffer BR before use. Blood samples may vary in the number of leukocytes depending on the individual, age, health status of donor and storage time. Processing too many cells may lead to overloading of the glass filter membrane. Therefore, ensure that there are not more than 5×10^6 leukocytes in the samples. If sample volume is less than 400µl, add the same amount of Buffer BR but adjust the total volume to 880µl with RNase-free Water. If sample volume is more than 400µl, adjust the volume of Buffer BR and RNase-free water proportionally.

2. **Separation from cell debris**

Centrifuge the sample at maximum speed for 3 min.

3. **Loading to Homogenization Column**

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

4. **Addition of ethanol**

Add 0.5 volumes of 80% ethanol to the flow-through. Mix thoroughly by pipetting.

If the flow-through is more than 900µl, separate it into two tubes before the addition of 0.5 volumes of 80% ethanol.

5. **Loading to RNA Binding Column**

Attention! Tips for Higher Yield

In order to obtain maximum yield, we strongly recommend users to fix the orientation of the column during centrifugation at all times. We recommend users to place the column which has a triangle mark on the edge, at a fixed position during centrifugation.

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

6. **Column washing 1**

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

Ensure that ethanol has been added into Wash Buffer before use (refer to Reconstitution of Solutions).

7. **DNase I digestion**

Pipette 70µl of **DNase I Digestion Mix** into **RNA Binding Column** and incubate at room temperature for 15 min.

Some samples may have high genomic DNA content. Prolong the incubation time (to be determined empirically) if necessary.

8. **Inhibitor removal**

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

(Ensure that ethanol has been added into Inhibitor Removal Buffer before use (refer to Reconstitution of Solutions)).

9. **Column washing 2**

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

Ensure that ethanol has been added into Wash Buffer before use (refer to Reconstitution of Solutions).

10. **Column drying**

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

It is essential to remove traces of ethanol as it will inhibit downstream applications.

11. **RNA elution**

Place the column into a clean 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.

Ensure that RNase-free Water is dispensed directly onto the center of the membrane for complete.

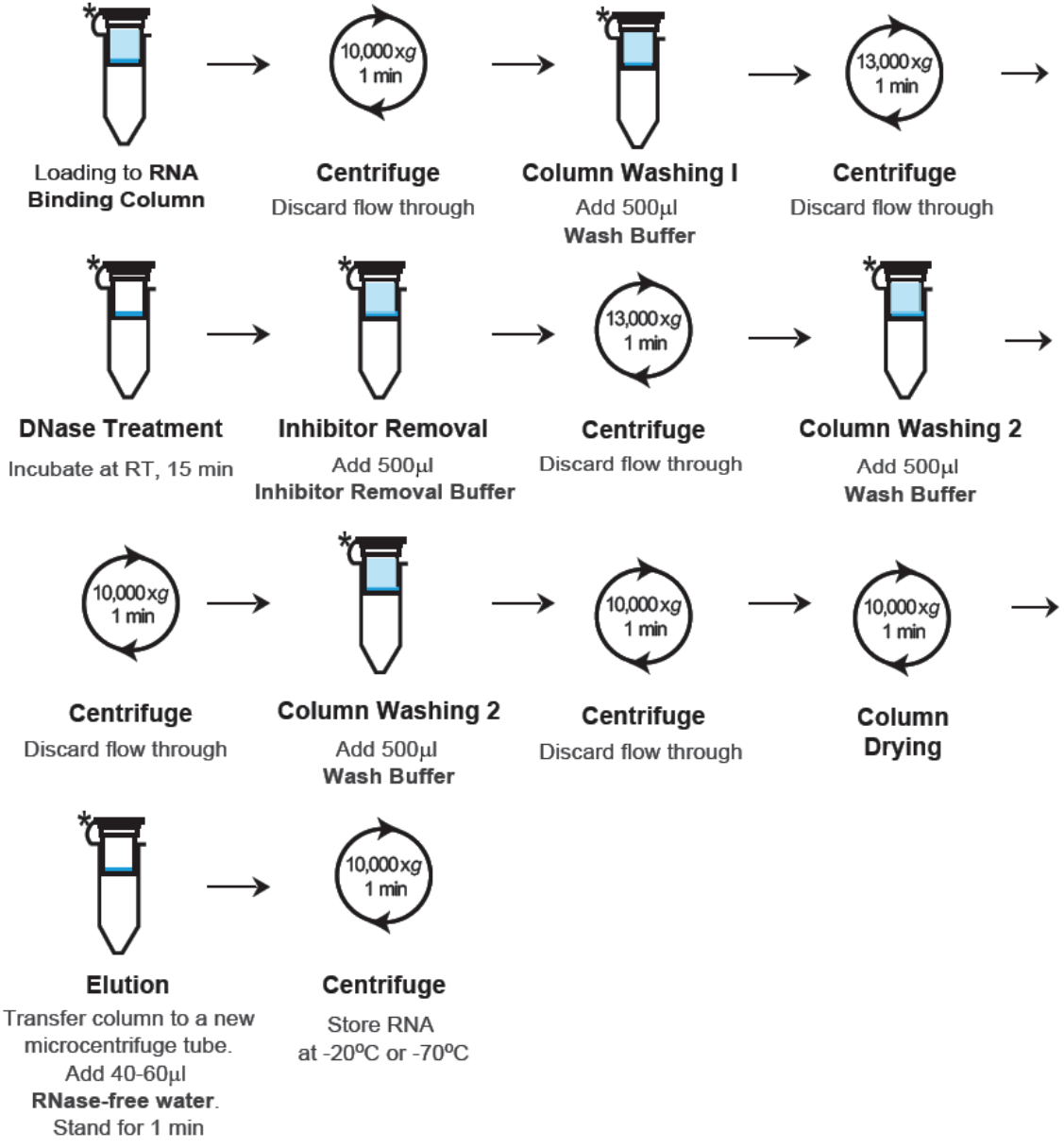
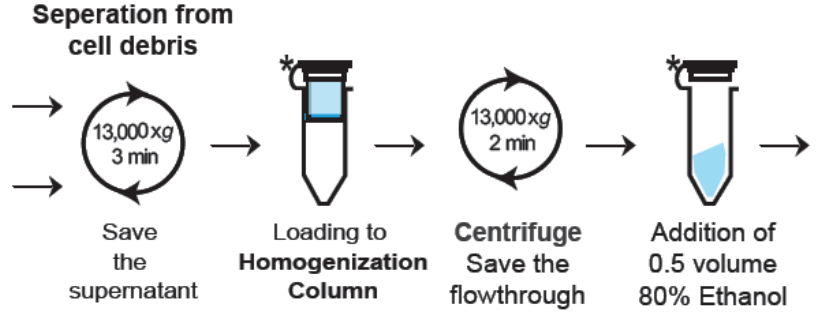
Troubleshooting

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

Problem	Possibility	Suggestions
Homogenization Column Clogged	<i>Too much starting material</i>	<i>Reduce amount of starting material in the subsequent purification.</i>
	<i>Lysate is too viscous</i>	<i>Users may dilute homogenate with additional lysis buffer.</i>
Low RNA yield	<i>Column is not placed at fixed orientation during centrifugation</i>	<i>Place the column which has a triangle mark on the edge, at a fixed position during centrifugation at all times.</i>
	<i>80% ethanol is not added prior loading onto RNA Binding Column</i>	<i>Repeat purification with new sample.</i>
	<i>80% ethanol is prepared wrongly</i>	<i>Repeat purification with new sample.</i>
	Inhibitor Removal Buffer and Wash Buffer are reconstituted wrongly	<i>Please refer to "Reconstitution of Solutions". Repeat purification with new sample.</i>
RNA degradation/ smearing	<i>Samples not properly stored</i>	<i>Fresh blood sample should be stored at 4°C and processed within a few hours of collection.</i>
		<i>If not processed immediately, store the sample at -70°C</i>
	<i>Inappropriate handling</i>	<i>Frozen blood sample should be thawed on ice.</i>
		<i>Use disposable plasticware and plastic tips.</i>

Problem	Possibility	Suggestions
Genomic DNA contamination		<i>Ensure that the purification is performed in an RNase-free environment.</i>
		<i>Wear gloves at all times.</i>
	<i>80% ethanol is not added prior loading onto RNA Binding Column</i>	<i>Repeat purification with new sample.</i>
	<i>80% ethanol is prepared wrongly</i>	<i>Repeat purification with new sample.</i>
Poor performance of eluted RNA in downstream applications	<i>Lysate is not passed through Homogenization Column prior loading onto RNA Binding Column</i>	<i>Repeat purification with new sample.</i>
	<i>DNase I digestion is not performed properly.</i>	<i>Please refer to preparation of DNA I Digestion Mix and pipette the DNase I Digestion Mix directly onto the membrane.</i>
	<i>Eluted RNA contains traces of ethanol</i>	<i>Ensure that the column is spun dried prior to elution.</i>
	<i>RNA degraded</i>	<i>Please refer to problem "RNA degradation/smearing".</i>

Blood Lysis
 Add 400µl blood into 300µl **Buffer BR** in a centrifuge tube. Mix by pulse-vortexing. Add 180µl **RNase-free water** and 20µl **Proteinase K**. Mix by pulse-vortexing. Incubate at 65°C, 10 min.



*** We recommend users to place the column which has a triangle mark on the edge, at a fixed position during centrifugation.**