Nucleic Acid Extraction Kit Handbook

GF-1

BLOOD TOTAL RNA EXTRACTION KIT USER GUIDE
(Version 2.1)

Catalog No.
SAMPLE: 5 preps
GF-TB-025: 25 preps
GF-TB-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 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	100 preps GF-TB-100
Components			
Homogenization Columns	5	25	100
RNA Binding Columns	5	25	100
Collection tubes	10	50	200
Buffer BR*	2ml	10ml	40ml
Inhibitor Removal Buffer*	1.5ml	8ml	30ml
Wash Buffer*	3ml	15ml	2x30ml
Proteinase K*	0.11ml	0.55ml	2x1.05ml
DNase I*	0.04ml	0.2ml	0.8ml
Digestion Buffer	0.35ml	2ml	8ml
Digestion Enhancer	0.04ml	0.2ml	0.8ml
RNase-free Water	1.ml	10ml	30ml
Handbook	1	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**.

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**.

For **GF-TB-100 (100 preps)**
Add **30ml** of absolute ethanol into the bottle labeled **Inhibitor Removal Buffer.**
Add **70ml** of absolute ethanol into the bottle labeled **Wash Buffe**r.
Add **70ml** of absolute ethanol into the bottle labeled **Wash Buffer** only prior to use.

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 18 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 $5x10^6$ 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 ~650µl of the lysate into a **Homogenization Column** assembled in a collection tube. Centrifuge at maximum speed for 2 min. Save the flow-through onto a clean 1.5ml microcentrifuge tube. Repeat for the remaining lysate from step 2.

4. Addition of ethanol

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

5. Loading to RNA Binding Column

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 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

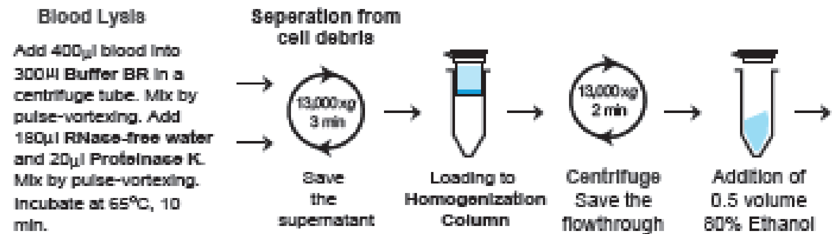
Place the column into a clean microcentrifuge tube. Add 40-60µl of **RNase-free Water** directly onto the membrane and stand for 1 min. Centrifuge at 10,000 x g for 1 min.
Store RNA at -20°C or -80°C.
Ensure that RNase-free Water is dispensed directly onto the center of the membrane for complete.

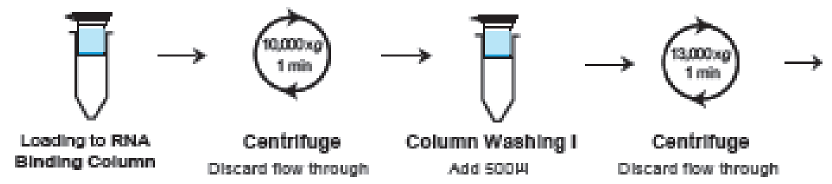
Troubleshooting

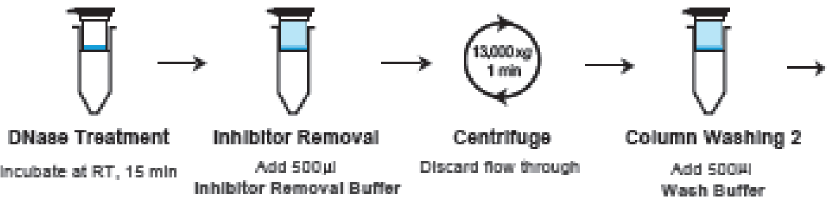
Please note that by not adhering to the recommended protocols, unsatisfactory results related to yield and quality of DNA may occur. If problems arise, please refer to the following:

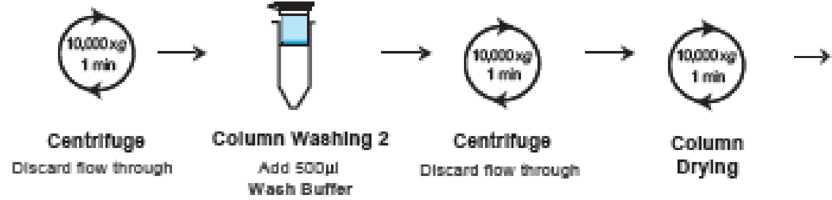
Problem	Possibility	Suggestions
Homogenization Column Clogged	*Too much starting material*	*Reduce amount of starting material in the subsequent purification.*
	Lysate is too viscous	*Users may dilute homogenate with additional lysis buffer.*
Low RNA yield		
	80% ethanol is not added prior loading onto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

Problem	Possibility	Suggestions
		Ensure that the purification is performed in an RNase-free environment. *Wear gloves at all times.*
Genomic DNA contamination	*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 *digestion is not performed properly.*	*Please refer to preparation of* ***DNase 1 Digestion Mix*** *and pipette the* ***DNase 1 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".*


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.
Seperation from cell debris
13,000xg 3 min
Save the supernatant
Loading to Homogenization Column
13,000xg 2 min
Centrifuge Save the flowthrough
Addition of 0.5 volume 80% Ethanol


10,000xg 1 min
13,000xg 1 min
Loading to RNA Binding Column
Centrifuge
Discard flow through
Column Washing I
Add 500μl Wash Buffer
Centrifuge
Discard flow through


13,000xg 1 min
DNase Treatment
Incubate at RT, 15 min
Inhibitor Removal
Add 500μl Inhibitor Removal Buffer
Centrifuge
Discard flow through
Column Washing 2
Add 500μl Wash Buffer


10,000xg 1 min
10,000xg 1 min
10,000xg 1 min
Centrifuge
Discard flow through
Column Washing 2
Add 500μl Wash Buffer
Centrifuge
Discard flow through
Column Drying

10,000xg 1 min
Elution
Transfer column to a new microcentrifuge tube. Add 40-60μl RNase-free water. Stand for 1 min
Centrifuge
Store RNA at -20°C or -70°C