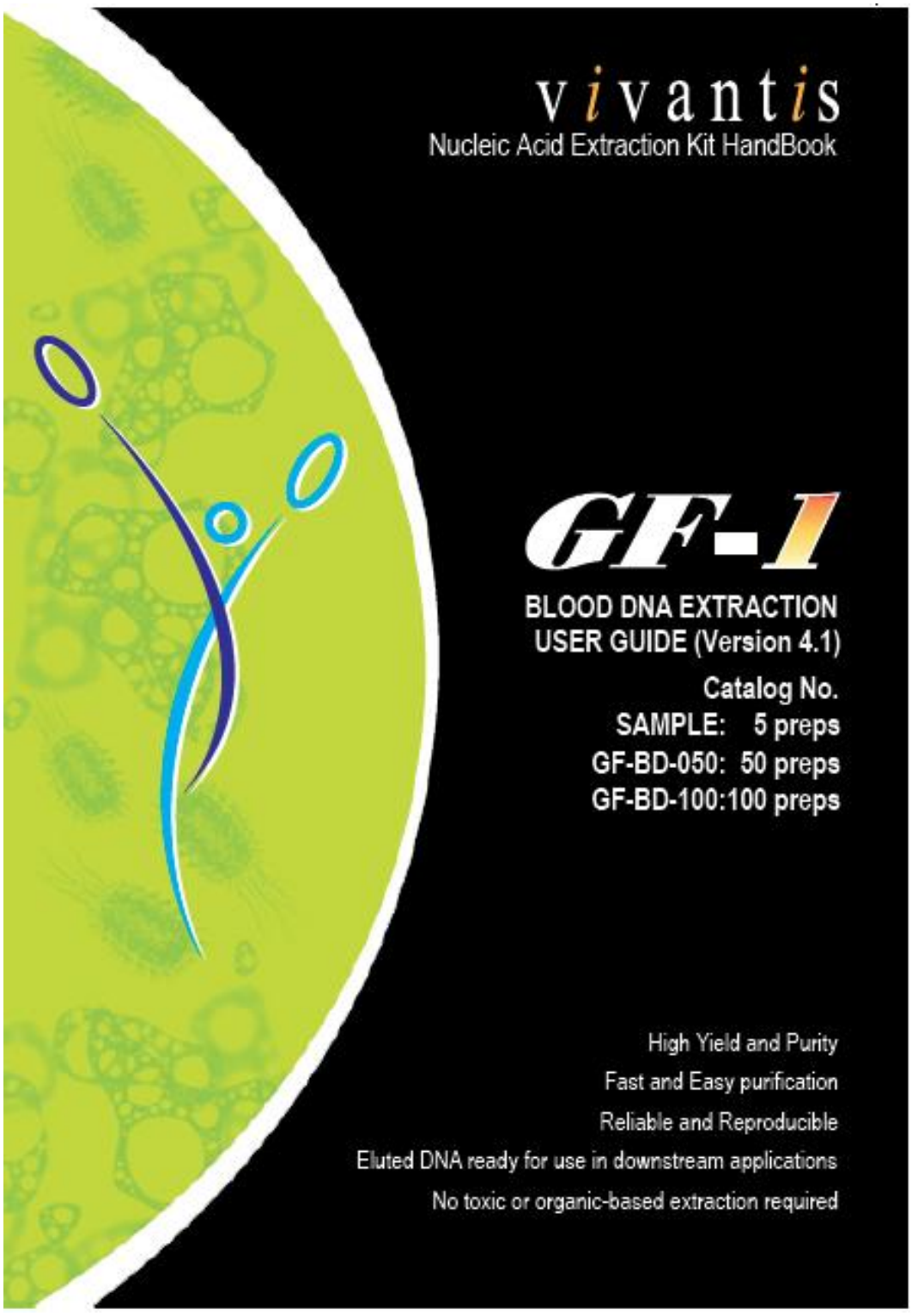
vivantis

Nucleic Acid Extraction Kit HandBook



GF-1

BLOOD DNA EXTRACTION USER GUIDE (Version 4.1)

Catalog No.
SAMPLE: 5 preps
GF-BD-050: 50 preps
GF-BD-100:100 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 DNA Extraction Kit** is designed for rapid and efficient purification of genomic DNA from up to 400µl whole blood. This kit uses a specially treated glass filter membrane fixed into a column to efficiently bind DNA in the presence of high salt. This kit applies the principle of a mini-column spin technology and the use of optimized buffers to ensure that only DNA is isolated while cellular proteins, metabolites, salt and other low molecular weight impurities are removed during the subsequent washing steps.

High-purity genomic DNA is then eluted in water or low salt buffers and has an $A_{260/280}$ ratio between 1.7 and 1.9 making it ready to use in many routine molecular biology applications such as restriction enzyme digestion, PCR, Southern blotting, DNA fingerprinting, and other manipulations.

Kit components

Product Catalog No	5 Preps SAMPLE	50 Preps GF-BD-050	100 Preps GF-BD-100
Components			
GF-1 columns	5	50	100
Collection tubes	5	50	100
Blood Lysis Buffer **(Buffer BB)**	1.5ml	12ml	24ml
Wash Buffer 1 (concentrate)*	1.5ml	15ml	30ml
Wash Buffer 2 (concentrate)*	2.4ml	17ml	34ml
Elution Buffer	1.5ml	10ml	20ml
Proteinase K*	0.11ml	1.05ml	2 x 1.05 ml
Handbook	1	1	1

* Please refer to **Reconstitution of Solutions** and **Storage and Stability** before using this kit.

The **GF-1 Blood DNA Extraction Kit** is available as 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%)
RNase A (DNase-free) (20mg/ml)

Reconstitution of Solutions

The bottle labeled **Wash Buffer 1** and **Wash Buffer 2** 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 **Wash Buffer 1**.
Add **5.6ml** of absolute ethanol into the bottle labeled **Wash Buffer 2.**

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

Add **15ml** of absolute ethanol into the bottle labeled **Wash Buffer 1**.
Add **40ml** of absolute ethanol into the bottle labeled **Wash Buffer 2**.

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

Add **30ml** of absolute ethanol into the bottle labeled **Wash Buffer 1**.
Add **80ml** of absolute ethanol into the bottle labeled **Wash Buffer 2**.
Store **Wash Buffer** at room temperature with bottle capped tight after use.

Storage and Stability

Store all solutions at 20°C - 30°C.
Proteinase K is stable for up to 1 year after delivery when stored at room temperature or 4°C. To prolong the lifetime of Proteinase K, storage at -20°C is recommended.
Kit components are guaranteed to be stable for 18 months from the date of manufacture **Buffer BB** may exhibit salt precipitation due to cold temperature. If this occurs, simply warm the bottle at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

Chemical Hazard

Buffer BB and **Wash Buffer 1** 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 other 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

Reminder

- All steps are to be carried out at room temperature unless stated otherwise.
- **Wash Buffer 1** and **Wash Buffer 2** (concentrate) have to be diluted with absolute ethanol before use. Please refer to **Reconstitution of Solutions**.
- If precipitation forms in **Buffer BB**, incubate at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

Pre-set waterbath to 65°C.
Pre-heat **Elution Buffer** at 65°C.

1. Blood lysis

Add 200µl of **Bufer BB** into a 200µl blood sample in a microcentrifuge tube. Mix thoroughly by pulsed-vortexing. Add 20µl of **Proteinase K** and mix immediately. Incubate at 65°C for 10 min.

Ensure that the Buffer BB is mixed homogeneously with blood sample before addition of Proteinase K. Blood samples may vary in the number of leukocytes depending on the donor. Processing too many cells may lead to overloading of the column. Therefore, ensure that there are not more than $5x10^6$ leukocytes in your sample. Users are not recommended to use more than 400µl sample. If the sample volume is more than 200µl, adjust the volume of buffers and Proteinase K to be added proportionately.

Optional: Removal of RNA

If RNA-free DNA is required, add 20µl of RNase A (DNase-Free, 20mg/ml). Mix and incubate at 37°C for 10 min.

2. Addition of ethanol

Add 200µl of absolute ethanol. Mix immediately and thoroughly to obtain a homogeneous solution.

Mix immediately to prevent any uneven precipitation of nucleic acids due to high local ethanol concentrations.

3. Loading to column

Transfer the sample into the column assembled in a clean collection tube (provided). Centrifuge at 5,000 x *g* for 1 min. Discard flow through.

4. Column washing 1.

Wash the column with 500µl **Wash Buffer 1** and centrifuge at 5,000 x *g* for 1 min. Discard flow trough.

Ensure that ethanol has been added into the Wash Buffer 1 before use (Refer to Reconstitution of Solutions)

5. Column washing 2

Wash the column with 500µl **Wash Buffer 2** and centrifuge at 5,000 x *g* for 1 min. Discard flow through. Wash column again with 500µl **Wash Buffer 2** and centrifuge at maximum speed for 3 minutes.

Ensure that ethanol has been added into the Wash Buffer 2 before use (refer to Reconstitution of Solutions).Ensure centrifugation for 3 min to remove ethanol completely.

6. DNA elution

Place the column into a clean microcentrifuge tube. Add 100µl of preheated **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 2 min. Centrifuge at 5,000 x *g* for 1 min to elute DNA. Store DNA at 4°C or 20 °C.

Ensure that the Elution Buffer is dispensed directly onto the center of membrane for complete elution. TE Buffer can also elute DNA although EDTA may inhibit subsequent enzymatic reactions. If water is used for eluting DNA, maximum elution efficiency is achieved between pH7.0 and 8.5.Store DNA at -20 °C as DNA may degrade in the absence of buffering agent.

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
Low DNA yield	*Blood sample is not stored properly*	*Store blood sample in small aliquots to avoid repeated freeze-thaw cycles.*
	Incomplete blood lysis	*Ensure* **Buffer BB** *and blood sample are mixed by pulsed-vortexing before addition of* **Proteinase K**.
		Ensure **Proteinase K** *is mixed homogeneously with the mixture of* **Buffer BB** *and blood sample.*
	Addition of ethanol was neglected	*Repeat purification with new blood sample.*
	Column clogged	*Please refer to the suggestion for 'Incomplete blood lysis.*
		Ensure **Wash Buffer 1** *is applied to the column.*
	Column not placed at fixed orientation during centrifugation	*Place the column which has a triangle mark on the edge, at a fixed position during centrifugation at all times.*
	Proteinase K *activity is decreased*	*For long term storage, ensure* **Proteinase K** *is stored at 4*°C or -20°C.
	Wash Buffer 1 and **Wash Buffer 2** *are applied in wrong order*	*Ensure* **Wash Buffer 1** *is applied before* **Wash Buffer 2.** *Repeat purification with a new blood sample.*

Problem	Possibility	Suggestions
	Wash Buffer 1 and **Wash Buffer 2** are reconstituted wrongly	*Please refer to the 'Reconstitution of Solution'. Repeat purification with a new blood sample.*
	Column is not dried before addition of **Elution Buffer**	*Ensure column is spun dry at maximum speed for 3 minutes after addition of* **Wash Buffer 2.**
	Elution is not performed properly	*Pre-heat* ***Elution Buffer*** *to 65°C-70°C for eluting DNA.*
		Incubate column at room temperature for 2 minutes after addition of **Elution Buffer**.
		Ensure that the **Elution Buffer** *used is a low salt buffer or water with a pH range of 7.0 - 8.5.*
Low Purity ($A_{260/280}$)	*Incomplete blood lysis*	*Refer to problem "Low DNA yield".*
	Proteinase K *activity is decreased*	*Refer to problem "Low DNA yield"*
	Wash Buffer 1 *is not applied*	*Ensure that* **Wash Buffer 1** *is applied to the column before addition of* **Wash Buffer 2.**
DNA degradation / smearing	*Incomplete blood lysis*	*Refer to problem "Low DNA yield"*
	Blood sample is not stored properly	*Refer to problem "Low DNA yield".*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Centrifuge the column at maximum speed for 3 min during second washing of column with* **Wash Buffer 2.**
	TE buffer is used to elute DNA. EDTA in TE buffer may inhibit subsequent enzymatic reaction	*Use* ***Elution Buffer*** *or water with a pH range of 7.0-8.5.*

Blood Lysis

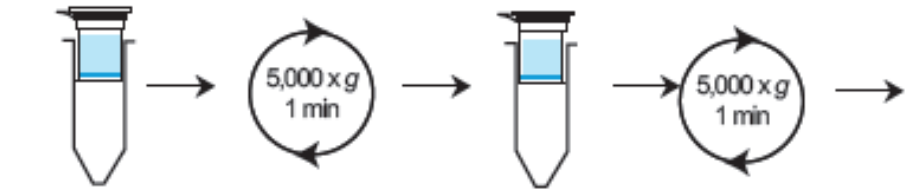
Add 200µl of Buffer BB into 200µl of blood in a centrifuge tube. Mix by pulsed vortexing. Add 20µl of Proteinase K. Mix homogeneously by pulsed-vortexing. Incubate at 65°C,10 min

Optional: Removal of RNA

Add 20µl of RNase A. Incubate 37°C, 5 min.

Addition of ethanol

Add 200µl absolute ethanol and mix immediately.



Loading to column

Transfer sample to column

Centrifuge

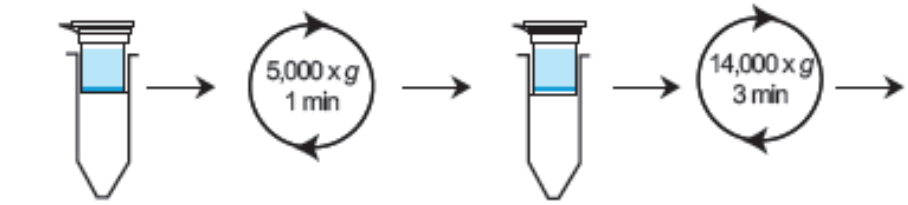
Discard flow through

Column Washing

Add 500µl Wash Buffer 1

Centrifuge

Discard flow through



Column Washing

Add 500µl Wash Buffer 2

Centrifuge

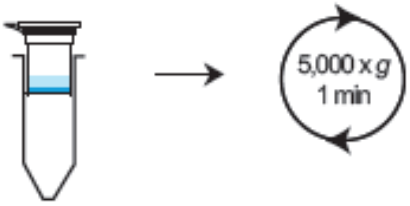
Discard flow through

Column Washing

Add 500µl Wash Buffer 2

Centrifuge

Discard flow through



Elution

Transfer column to a new microcentrifuge tube

Add 100µl preheated Elution Buffer or water. Stand for 2 min.

Centrifuge

Store DNA at 4°C or -20°C

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