GF-1 Blood Starter Kit / *Taq* DNA Polymerase

- GF-1 Blood DNA Extraction kit
- GF-1 Gel DNA Recovery Kit
- DNA Amplification Kit

Product Code: GF-BD-K
Pack Size: 25 preps

GF-1 BLOOD DNA EXTRACTION KIT
Catalogue No.: GF-BD-K, 25 preps

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	25 Preps
Catalog No	GF-BD-K
Components	
GF-1 columns	25
Collection tubes	25
Blood Lysis Buffer **(Buffer BB)**	6ml
Wash Buffer 1 (concentrate)*	8ml
Wash Buffer 2 (concentrate)*	17ml
Elution Buffer	10ml
Proteinase K*	0.52ml
Handbook	1

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

Add **8ml** of absolute ethanol into the bottle labeled **Wash Buffer 1**.
Add **40ml** of absolute ethanol into the bottle labeled **Wash Buffer 2**.

Store **Wash Buffer 1** and **Wash Buffer 2** at room temperature with bottle capped tight after use.

Storage and Stability

Store all solutions at 20°C - 30°C.
Store **Proteinase K** at -20°C.
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*	*Ensure* **Proteinase K** *is stored at -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.*

DNA AMPLIFICATION KIT
Catalogue No.: GF-BD-K, 25 preps

INTRODUCTION

The DNA Amplification Kit is designed to contain high quality *Taq* DNA Polymerases, buffers, dNTPs mix, and nuclease-free water as a complete set for user's convenience in performing DNA amplification experiments. The kit allows for up to 50 applications or more in a 50µl PCR reaction. DNA and primers are also provided as a positive control for users to carry out PCR using the recommended parameters as shown in this manual.
The kit is also supplied with DNA ladders (ready-to-use) for up to 50 applications to determine the size of PCR products or other double-stranded DNA fragments during gel electrophoresis.

KIT COMPONENTS

PCR Amplification Reagents	
Taq DNA Polymerase (5u/µl)	200u
10X ViBuffer A (500mM KCl, 100mM Tris-HCl (pH 9.1) and 0.1% Triton X-100)	1ml
10X ViBuffer S (160Mm $(NH_4)_2SO_4$, 500mM Tris-HCl (pH9.2), 17.5mM $MgCl_2$ and 0.1% Triton X-100)	1ml
50mM $MgCl_2$	1ml
2mM dNTPs Mix	0.25ml
Control DNA (5ng/µl)	100ng
10µM Forward primer	25µl
10µM Reverse primer	25µl
Nuclease-free water	1ml X 2
DNA Ladder (ready-to-use)	
VC 100bp plus (0.1µg/µl)	50 applications
VC 1kb (0.1µg/µl)	50 applications
6X loading dye	100µl

STORAGE & STABILITY

Store all components at -20°C
Kit components are guaranteed to be stable for 2 year from the date of manufacture.

PCR PROTOCOL (Control DNA)

1. Gently mix all solutions after thawing. Keep solutions on ice from this point onwards.
2. Add the following reagents into a PCR tube, on ice.

Reagent	Quantity (µl)	Final Concentration
Water, nuclease-free	38.1	-
10X ViBuffer A	5.0	1X
2mM dNTPs mix	2.0	0.08mM
50mM $MgCl_2$	1.5	1.5mM
10uM Forward Primer	1.0	0.2µM
10uM Reverse Primer	1.0	0.2µM
Control DNA (5ng/µl)	1.0	5ng
Taq DNA Polymerase (5u/µl)	0.4	2unit
Total Volume	50.0	

3. Gently mix the PCR reagents. Briefly centrifuge the tubes to collect the contents at the bottom of the tube.
4. Perform DNA amplification using the following program:

Segment	No. of cycles	Temperature	Duration
1	1	95°C	3 min
2	30	95°C	30 sec
		52°C	30 sec
		72°C	30 sec
3	1	72°C	5 min
	1	4°C	pause

5. Run 5µl of the PCR products along with 0.3 – 0.5µg of VC100bp plus DNA ladder in a 1.0% agarose gel. Stain gel with EtBr to visualize DNA bands under UV.

Note: The PCR product of the positive control should provide a 1.4kb DNA fragment size.

TROUBLESHOOTING

PCR Reactions

Problems	Possibility	Suggestions
Low yield or no PCR product	Missing component in reaction	Check the reaction components and repeat the reaction.
Multiple, non-specific amplification products	Cross contamination of DNA	Use a separate workplace and pipettes for PCR. Wear gloves at all times.
	Excessive amounts of enzyme used	Decrease amount of *Taq* DNA Polymerase in the reaction tube.
	Excessive amounts of DNA template used	Decrease amount of DNA template in the reaction tube.
	Excessive number of cycles	Reduce number of cycles.
	Excessive amount of $MgCl_2$ used	Decrease the concentration of $MgCl_2$ in the reaction tube.
	Long extension time	Reduce extension time.
	Pipetting error	Perform PCR in reaction master mixes.
Smearing of PCR product when viewed after gel electrophoresis	Agarose gel used was not fresh	Repeat electrophoresis with fresh agarose gel.
	Insufficient amount of $MgCl_2$ used	Increase the concentration of $MgCl_2$ in the reaction tube.
	Excessive amounts of enzyme used	Decrease amount of *Taq* DNA Polymerase in the reaction tube.

GF-1 GEL DNA RECOVERY KIT
Catalogue No.: GF-BD-K, 25 preps

Introduction

The **GF-1 Gel DNA Recovery Kit** is a system designed for rapid purification of DNA bands ranging from 100bp to 10kb from all grades of agarose gel in TAE (Tris-acetate/ EDTA) or TBE (Tris-borate/ EDTA) buffer. The Gel DNA Binding Buffer (**Buffer GB**) is optimized to enhance binding of DNA onto a specially-treated glass filter membrane at pH7.0 or below. High recovery of pure DNA is obtained and ready to use in many routine molecular biology applications such as restriction enzyme digestion, radioactive/ fluorescence DNA sequencing, PCR, ligation, probe preparations and other manipulations.

Kit components

Product	25 Preps
Catalog No.	GF-GP-K
Components	
GF-1 columns	25
Collection tubes	25
Gel DNA Binding Buffer (Buffer GB)	15ml
Wash Buffer (concentrate)*	17ml
Handbook	1

* Please refer to **Reconstitution of Solutions** and **Storage and Stability** before using this kit.
The Elution Buffer is applicable for both **Blood DNA Extraction Kit and **Gel DNA Recovery 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

Reconstitution of Solutions

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

Add **40ml** of absolute ethanol into the bottle labeled **Wash Buffer**. Once diluted, the **Wash Buffer** is applicable for both **Blood DNA Extraction Kit** and **Gel DNA Recovery Kit.**

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

Storage and Stability

All solutions should be stored at 20°C - 30°C.

Kit components are guaranteed to be stable for 18 months from the date of manufacture.

Buffer GB 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 GB 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 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 (concentrate) has to be diluted with absolute ethanol before use. Please refer to **Reconstitution of Solutions.**

If precipitation forms in **Buffer GB**, incubate at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

The amount of **Buffer GB** provided is sufficient for each purification of 0.5g of agarose DNA gel. In the case of inadequate amounts of **Buffer GB,** please make a separate purchase for additional buffer as required.

Pre-set waterbath to 50°C.

1. Gel Electrophoresis

Run DNA sample on agarose gel electrophoresis to separate DNA fragments. Perform ethidium bromide staining for DNA visualization. Cut agarose gel band containing the desired DNA and place it into a pre-weighed microcentrifuge tube.

Ensure that the electrophoresis run is sufficient before performing excision of DNA fragment. Avoid more than 30 sec exposure of UV light onto the DNA.

2. Solubilization of agarose

Determine the nett weight of gel slice and add 1 volume of **Buffer GB** to 1 volume of gel (*A gel slice of mass 0.1g will have a volume of 100µl)*. Centrifuge the tube briefly to make sure the gelslice stays at the bottom of the tube. Incubate at 50°C until gel has melted completely. Mixoccasionally to ensure complete solubilization.

3. Loading to column

Transfer the sample into a column assembled in a clean collection tube (provided). Centrifuge at 10,000 x *g* for 1 min. Discard flow through. Repeat for any remaining sample from step 2.

4. Column washing

Add 650µl **Wash Buffer** into the column. Centrifuge at 10,000 x *g* for 1 min. Discard flow through.

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

5. Column drying

Centrifuge the column at 10,000 x *g* for 1 min to remove residual ethanol.

This step has to be carried out to remove all traces of ethanol as residual ethanol can affect the quality of DNA and may subsequently inhibit enzymatic reactions.

6. DNA elution

Place the column into a clean microcentrifuge tube. Add 30 - 50µl **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 2 min. For DNA fragments larger than 8kb, use preheated elution buffer at 65°C - 70°C for better elution efficiency. Centrifuge at 10,000 x *g* for 1 min to elute DNA. Store DNA at 4°C or -20°C.

For higher yield, elute DNA in 50µl and for higher concentration, elute DNA in smaller volume, i.e: 30µl. However, the yield will be slightly reduced. Ensure that the elution buffer is dispensed directly onto the center of the 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 a 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
Gel slice does not dissolve	*High percentage gel used*	*Extend incubation time with mixing until the gel has completely dissolved.*
	Gel slice is too big	*Minimize gel size by removing extra gel and slice the gel into smaller pieces to enhance solubilization.*
Low recovery of DNA	*Incomplete DNA elution*	*Allow full contact of* **Elution Buffer** *with membrane by dispensing directly onto the center of the membrane. Do not elute with less than 30µl of elution buffer.*

	TAE or TBE buffer repeatedly used or pH incorrect	*pH of repeatedly used TAE or TBE buffer normally increases. Preferably, use fresh TAE or TBE buffer for each gel electrophoresis run.*
	DNA diffused or released into buffer during electrophoresis, staining and destaining.	*Minimize DNA migration distance during electrophoresis. Do not overlay gel with too much buffer during loading of sample. Minimize staining and destaining time.*
Low recovery of DNA smaller than 400bp	*Elevated temperatures may cause denaturation of DNA into ssDNA*	*Solubilize agarose at 40°C instead of 50°C for an extended period with repeated mixing.*
	Binding efficiency reduced due to small DNA size	*Add 1 gel volume of isopropanol to sample prior to loading onto column.*
Low recovery of DNA larger than 8kb	*Low elution efficiency*	*Pre-heat* **Elution Buffer** *to 65°C - 70°C before eluting DNA.*
	Binding efficiency reduced due to large DNA size	*Add 1 gel volume of isopropanol to sample prior to loading onto column.*
No DNA eluted	*Inappropriate elution buffer*	*Ensure that the elution buffer used is a low salt buffer or water with a pH range of 7.0 - 8.5.*
Non-specific DNA fragments appears in eluted DNA	*Migration distance insufficient during electrophoresis*	*Ensure that the electrophoresis run is sufficient to separate bands before performing cut.*
	Scalpel or razor blade used to excise the gel is contaminated with other DNA fragments	*Use a new or clean scalpel or razor blade to excise the gel.*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Ensure that the Column drying step is carried out prior to elution.*
	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.*