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

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

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

GF-1 BACTERIAL DNA EXTRACTION KIT
Catalogue No.: GF-BA-K, 25 preps

Introduction

The **GF-1 Bacterial DNA Extraction Kit** is designed for rapid and efficient purification up to 20µg of a high molecular weight genomic DNA from either Gram-negative or Gram –positive bacteria. 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, salts and other low molecular weight impurities are removed during the subsequent washing steps. High-purity genomic DNA is eluted in water or low salt buffers and has a $A_{260/280}$ ratio between 1.7 and 1.9 making it ready-to-use in many routine molecular biology applications such as restriction enzyme digestion, Southern blotting, PCR,DNA fingerprinting and other manipulations.

Kit component

Product	25 Preps
Catalog No.	GF-BA-K
Components	
GF-1 columns	25
Collection tubes	25
Resuspension Buffer 1 **(Buffer R1)**	4ml
Resuspension Buffer 2 **(Buffer R2)**	5ml
Bacterial Genomic Binding Buffer (**Buffer BG)**	14ml
Wash Buffer	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

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Note: The **GF-1 Bacterial DNA Extraction Kit** is optimized to isolate up to 20µg of DNA on recommended cell culture volume. Bacteria cultures vary in the number of cells depending on the strain, growth conditions and viability of the cells. When processing samples, do not use more than the recommended starting volume as it will lead to excessive number of cells and overloading of the column. This would result on reduced yield and purity. The recommended cell culture volume is between 1 to 3ml for both Gram-negative and Gram-positive bacteria to ensure yield and purity is obtained.

Additional Materials to be Supplied by User
Absolute Ethanol (>95%)
Lysozyme (50mg/ml)
RNase A (DNase-free) (20mg/ml)

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 **Bacterial DNA Extraction Kit** and **Gel DNA Recovery Kit.**
Store **Wash Buffer** at room temperature with bottle capped tight after use.

Storage and Stability
Store 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 BG** 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 BG 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 BG**, incubate at 55°C - 65°C with occasional mixing until completely dissolved.

Pre-set waterbath to 37°C and the second waterbath to 65°C.
Pre-heat **Elution Buffer** to 65°C (optional).

1. Centrifugation

Pellet 1 - 3ml of bacteria culture grown overnight or culture grown to log phase by centrifugation at 6,000 x *g* for 2 min at room temperature. Decant the supernatant completely.

Thorough removal of supernatant is essential as residual culture media may affect both yield and purity.

2. Resuspension of pellet

Add 100µl **Buffer R1** to the pellet and **r**esuspend the cells completely by pipetting up and down.

Ensure complete cell resuspension. Lysis will not occur if clumps of bacteria remain following an inefficient resuspension procedure.

3. Lysozyme treatment

For Gram-negative bacteria strains, add 10µl lysozyme (50mg/ml) into the cell suspension.
For Gram-positive bacteria strains, add 20µl lysozyme (50mg/ml) into the cell suspension.
Mix thoroughly and incubate at 37°C for 20 min.

Some bacterial strains may require longer incubation time in lysozyme.

4. Centrifugation

Pellet digested cells by centrifugation at 10,000 x *g* for 3 min. Decant the supernatant completely.

5. Protein denaturation

Resuspend pellet in 180 µl of **Buffer R2** and add 20 µl of **Proteinase K**. Mix thoroughly. Incubate at 65°C for 20 min in a shaking waterbath or with occasional mixing every 5 min.

Lysate should be clear at the end of incubation or else extend the incubation time to 30 min.

Optional: Removal of RNA

If RNA-free DNA is required, add 20 µl of RNase A (DNase-free, 20mg/ml). Mix and incubate at 37°C for 5 min.

Residual RNA fragments will be removed during column washing.

6. Homogenization

Add 2 volumes (~400 µl without RNase A treatment, ~440 µl with RNase A treatment) of **Buffer BG** and mix thoroughly by inverting tube several times until a homogeneous solution is obtained.Incubate for 10 min at 65°C.

7. Addition of Ethanol

Add 200 µl of absolute ethanol. Mix immediately and thoroughly.

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

8. Loading to column

Transfer the sample (max.650µl) into a column assembled in a clean collection tube (provided). Centrifuge at 10,000 x *g* for 1 min. Discard flow through.

If column clogs, add 200µl Buffer BG into column and centrifuged as above.

9. Column washing

Wash the column with 650 µl of **Wash Buffer** and 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).

10. 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.

11. DNA elution

Place the column into a clean microcentrifuge tube. Add 50 - 100 µl of preheated **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 2 min. Centrifuge at 10,000x *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 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
Low DNA yield	*Incomplete cell resuspension*	*Ensure that cells are completelyresuspended in* **Buffer R1** *and* **Buffer R2** *before incubation in lysozyme and* **Proteinase K**.
	Low elution efficiency	*Pre-heat* **Elution Buffer** *to 65°C - 70°C before eluting DNA.*
	Column clogged	*Refer to Problems under 'Column clogged'.*

Low purity	*Incomplete protein denaturation*	*Ensure that cells are completely resuspended in* **Buffer R1** *and* **Buffer R2** *before incubation in lysozyme and* **Proteinase K**. *Extend incubation time until lysate clears.*
	RNA contamination	*Add RNase A to the sample as indicated in the protocol. Ensure that RNase used has not been repeatedly frozen and thawed. If necessary prepare a fresh stock.*
Difficult to resuspend cell pellet in Buffer R1 or R2	*Centrifugation at high speed and long periods*	*Ensure that the cell culture is centrifuged at the recommended speed and time.* *Using pipette tips, pipette the lysate up and down until completely homogenized.*
No DNA eluted	*Inappropriate elution buffer*	*Ensure that the* **Elution Buffer** *used is a low salt buffer or waterwith a pH range of 7.0-8.5.*
Lysate not clear after incubation with Buffer R2	*Lysozyme activity may decrease with time*	*Ensure that lysozyme is fresh and has not been repeatedly frozen and thawed.*
Column clogged	*Lysate is not clear due to insufficient digestion or lysis*	*Ensure that the lysate is clear prior to sample loading onto the column. If necessary, extend incubation time in lysozyme and* **Proteinase K**.
	Overloading of column / starting culture volume too high	*Use the recommended culture volume between 1 - 3ml.*
DNA degradation / smearing	*DNA sheared during purification*	*After the addition of* **Proteinase K**, *avoid vigorous mixing and pipetting. Mix gently by inverting tube.*
	Nuclease contamination	*Use sterilized glassware, plasticware and wear gloves. Ensure that cells are completely resuspended in* **Buffer R2**.
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.*

DNA AMPLIFICATION KIT
Catalogue No.: GF-BA-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-BA-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.*