GF-1 Plasmid Starter Kit / Chromo *Taq* DNA Polymerase

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

Product Code: GF-PL-KW
Pack Size: 25 preps

GF-1 PLASMID DNA EXTRACTION KIT
Catalogue No.: GF-PL-KW, 25 preps

Introduction

The **GF-1 Plasmid DNA Extraction Kit** is designed for rapid and efficient purification of high copy and low copy plasmid DNA without the need for precipitation or organic extractions. This it uses a specially-treated glass filter membrane fixed into a column to efficiently bind DNA in the presence of high salt. Combining alkaline lysis-SDS and mini-column spin technology, up to 20µg of plasmid DNA from bacterial cultures can be isolated. Multiple samples can be processed rapidly and with practice, the purification takes less than 30 minutes. Optimized buffers ensure only highly pure plasmid DNA is extracted and is ready to use in many routine molecular biology applications such as restriction enzyme digestion, radioactive/fluorescence DNA sequencing, PCR, ligation, transformation and other manipulations.

	Culture Vol.	**Yield**	**Purity**
High Copy Number Plasmid	2ml	2-5µg	1.7-1.9
(50-500 copies per cell)	5ml	10-20µg	1.7-1.9
Low Copy Number Plasmid	2ml	1-3µg	1.7-1.9
(1-10 copies per cell)	5ml	5-10µg	1.7-1.9

Kit component

Product	25 Preps
Catalog No.	GF-PL-KW
Components	
GF-1 columns	25
Collection tubes	25
Solution 1 **(Buffer S1)***	8ml
Solution 2 **(Buffer S2)***	8ml
Neutralizing Buffer **(Buffer NB)**	13ml
Wash Buffer (*concentrate)	17ml
Elution Buffer	10ml
RNase A (DNase Free)*	0.04ml
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%)

Reconstitution of Solutions

The bottle labeled **Wash Buffer** contains concentrated buffer which must be diluted with absolute ethanol (>95%) before use.
The vial of **RNase A (DNase-Free)** provided is to be added into the bottle labeled **S1**.

Add **40ml** of absolute ethanol into the bottle labeled **Wash Buffer**. Once diluted, the **Wash Buffer** is applicable for both **Plant DNA Extraction Kit** and **Gel DNA Recovery Kit.**
Add **1ml** of **S1** into the vial of **RNase A** and mix well. Briefly centrifuge and transfer the entire mixture back into the **S1** tube. Mix well.
Store **Wash Buffer** at room temperature with bottle capped tight after use.

Storage and Stability

Store solutions at 20°C – 30°C.
RNase A in **S1** should be stable for 6 months when the solution is stored at 2°C - 8°C.
Kit components are guaranteed to be stable for 18 months from the date of manufacture **Buffer NB** 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 NB 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

1. All steps are to be carried out at room temperature unless stated otherwise.
2. **Wash Buffer** (concentrate) has to be diluted with absolute ethanol and **S1** to be added with **RNase A** before use. Please refer to **Reconstitution of Solutions**.
3. If precipitation forms in **Buffer NB**, incubate at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

1. Preparation of stock culture

Grow 5 - 10ml plasmid-containing bacteria cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) at 37°C with agitation.

Fresh culture must always be used for extraction.

2. Centrifugation

Pellet 1.5 - 5ml of bacterial culture containing the plasmid by centrifugation at 6,000 x *g* for 2 min. If 15ml or 50ml centrifuge tube is used to harvest the cells, centrifuge at 6,000 x *g* for 5 min. Decant the supernatant completely.

Do not centrifuge cells at high speed or for long periods. Cells will become too compact for resuspension.

3. Resuspension of pellet

Add 250µl **S1** to the pellet and resuspend the cells completely by vortexing or pipetting. Transfer the suspension to a clean 1.5ml microcentrifuge tube.

Ensure that cells are completely resuspended. Lysis will not occur if clumps of bacteria remain following an inefficient resuspension procedure. Ensure that RNase A has been added into the S1 buffer before use (refer to Reconstitution of Solutions).

4. Alkaline lysis

Add 250µl of **S2** and **gently mix** by inverting tube several times (4-6 times) to obtain a clear lysate. Incubate on ice or at room temperature for **NOT** longer than 5 min.

Do NOT vortex! Vortexing shears the genomic DNA and leads to contamination with chromosomal DNA.

S2 should be immediately capped tightly after used. Incubation on ice may reduce non-supercoiled plasmid contamination in some bacteria strains. Precipitation of SDS and cell debris in the following Neutralization step will be slightly more effective in the cold.

5. Neutralization

To neutralize the lysate, add 400µl of **Buffer NB** and **gently mix** by inverting the tube several times (6-10 times) until a white precipitate forms. Centrifuge at 14,000 - 16,000 x *g* for 10 min.

*Do **NOT** vortex upon addition of Buffer NB! Vortexing shears the genomic DNA and leads to contamination with chromosomal DNA. After centrifugation, the compact white precipitate should be spun down and separated from the supernatant.*

6. Loading to column

Transfer 650µl of **supernatant** into a column assembled in a clean collection tube (provided).

Centrifuge at 10,000 x *g* for 1 min. Discard flow through.

Repeat for the remaining sample from step 5.

Be careful not to transfer any white precipitate into the column.

7. Column washing

Wash the column with 650µl **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).

8. 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

9. DNA elution

Place the column into a clean microcentrifuge tube. Add 50 - 100µl of **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 1 min. Centrifuge at 10,000 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 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	*Cell lysis incomplete / Lysate did not clear after addition of* **S2**	*Do not exceed the recommended culture volume of 5ml.*
		Use fresh **S2** *by preparing as follows: 0.2N NaOH, 1% SDS.*
	Poor resuspension of cells	*Ensure that cells are completely resuspended after the addition of* **S1**. *No cell clumps should be visible.*
	Low copy-number plasmid	*Increase culture volume or grow culture in enriched medium such as Terrific Broth to increase the yield.*
	Bacteria culture overgrown or not fresh	*Do not culture bacteria longer than 20 hours at 37°C as this may lower the plasmid yield. Media should contain antibiotic at an appropriate concentration.*

	Elution is not performed properly	*Ensure that the elution buffer used is a low salt buffer or water with a pH range of 7.0 - 8.5.*
Column clogged	*Transfer of precipitate from sample prior to loading into column*	*Ensure that white precipitate is not transferred over during loading of column to prevent clogging up of the membrane.*
High molecular weight DNA contamination.	*Vigorous mixing of lysate upon addition of* **S2** *or* **Buffer NB**	*Do not vortex or mix vigorously after addition of* **S2** *or* **Buffer NB**. *Simply mix by gently inverting the tube a few times.*
	Incubation longer than 5 min after addition of **S2**	*Do not incubate longer than 5 min.*
Additional plasmid formation	*Irreversible denaturation during cell lysis*	*Do not carry out incubation longer than 5 min after the addition of* **S2**.
	Nicked circular plasmids due to the presence of nucleases	*Carry out purification without delay at least until the washing step where nucleases will be removed.*
		Incubation on ice after addition of **S2** *reduces nuclease activity.*
RNA Contamination	*RNA digestion was insufficient*	*Ensure that* **RNase A** *has been added into* **S1** *or add a new preparation of* **RNase A** *into* **S1** *to a final concentration of 100µg/ml.*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Ensure that the Column drying step 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.*

DNA AMPLIFICATION KIT
Catalogue No.: GF-PL-KW, 25 preps

INTRODUCTION

The DNA Amplification Kit is designed to contain high quality Chromo *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	
Chromo *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	36.5	-
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
Chromo *Taq* DNA Polymerase (5u/µl)	2.0	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 Chromo *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 Chromo *Taq* DNA Polymerase in the reaction tube.

GF-1 GEL DNA RECOVERY KIT
Catalogue No.: GF-PL-KW, 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-KW
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

4. All steps are to be carried out at room temperature unless stated otherwise.
5. **Wash Buffer** (concentrate) has to be diluted with absolute ethanol before use. Please refer to **Reconstitution of Solutions.**
6. If precipitation forms in **Buffer GB**, incubate at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.
7. 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 gel slice stays at the bottom of the tube. Incubate at 50°C until gel has melted completely. Mix occasionally 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.*