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

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

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

GF-1 PLANT DNA EXTRACTION KIT
Catalogue No.: GF-PT-K, 25 preps

Introduction

The **GF-1 Plant DNA Extraction Kit** is designed for rapid and efficient purification of genomic DNA from a variety of plant tissues without the need for precipitation or organic extraction. This kit uses a specially-treated glass filter membrane fixed into a column to efficiently bind DNA in the presence of high salt. The kit applies the principle of a mini-column spin technology and the use of optimized buffers to ensure that only DNA is isolated while proteins and other impurities, additives, preservatives are removed during the subsequent washing steps. High purity genomic DNA is then eluted in water or low salt buffers 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, DNA fingerprinting, PCR and other manipulations

Kit component

Product	25 Preps
Catalog No.	GF-PT-K
Components	
GF-1 columns	25
Collection tubes	25
Plant Tissue Lysis Buffer **(Buffer PL)**	9ml
Plant Genomic Binding Buffer **(Buffer PB)**	18ml
Wash Buffer (*concentrate)	24ml
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

Reconstitution of Solutions

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

Add **56ml** 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.**
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 PB** 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 PB 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 PB**, incubate at 55°C - 65°C with occasional mixing until completely dissolved.

Pre-set waterbath to 65°C.

Pre-heat **Elution Buffer** at 65°C (optional).

1. Homogenization

Cut 10 - 30mg of tissue sample into small pieces with a clean scalpel. Freeze sample in liquid nitrogen and grind into a fine powder with a mortar and pestle.

The tissue sample should be ground into fine powder to ensure complete lysis in the next step.

2. Tissue Lysis

Add 280µl of **Buffer PL** to the ground sample. Mix thoroughly by vortexing the tube for 30 sec to obtain a homogeneous solution. Add 20µl of **Proteinase K** and mix thoroughly by inverting tube. Incubate at 65°C for 1 - 2 hrs (or overnight if tissue mixture does not appear clear) in a shaking waterbath or mix several times during incubation to ensure thorough digestion of the sample.

If tissue sample was not reduced to small pieces, homogenize sample in buffer with multiple strokes using a tube pestle. Solubilization of tissue sample varies between different tissue types. If insoluble materials still remain, extend incubation time or increase the amount of Proteinase K to ensure complete lysis.

3. Centrifugation

Centrifuge at 14,000 - 16,000 x *g* for 5 min to precipitate any insoluble/undigested materials. Transfer the supernatant containing the DNA into a clean microcentrifuge tube.

Extend centrifugation time if solids are not completely spun down.

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 5 min.

Residual RNA fragments will be removed during column washing.

4. Homogenization

Add 2 volumes (~600µl without RNase A treatment, ~640µl with RNase A treatment) of **Buffer PB** and mix thoroughly until a homogeneous solution is obtained by inverting tube several times. Incubate for 10 min at 65°C.

Precipitation may occur due to high DNA content. Lysate should be clear upon mixing and incubation at 65°C.

5. Addition of ethanol

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

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

6. Loading to column

Transfer the sample into a column (max. 650µl) 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 6.

If column clogs, add 200µl Buffer PB into column and centrifuge as above.

7. Column washing

Wash the column with 650µl **Wash Buffer** and centrifuge at 10,000 x *g* for 1 min. Discard flow through. Repeat washing if color stains from sample remains on the column membrane.
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 completely 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 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:

Problems	Possibility	Suggestions
Low DNA yield	*Sample not thoroughly homogenized*	*Ensure that tissues are completely homogenized in* **Buffer PL**
	Samples not fresh or not properly stored	*For long term storage of tissues, keep at* -70°C.
	Sample not lysed completely	*Ensure that tissues are completely homogenized in* ***Buffer PL*** *and mix sample frequently during incubation in absence of a waterbath shaker.*
	Low elution efficiency	*Pre-heat* **Elution Buffer** *to 65°C-70°C before eluting DNA.*

Low purity	*Incomplete protein denaturation*	*Extend incubation time until lysate clears.*
	RNA contamination	*Add RNase A to the sample as indicated in the protocol. Ensure that RNase A used has not been repeatedly frozen and thawed. If necessary prepare a fresh stock*
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*
Column clogged	*Overloading of column/ starting material too high*	*Do not use more than 30mg of sample material. If any undigested material remain, spin to remove tissue lysate and transfer supernatant into a new microcentrifuge tube.*
	Sample not thoroughly homogenized	*Vortex sample in **Buffer PL** prior t addition of **ProteinaseK***
DNA degradation/ smearing	*DNA sheared during purification*	*After the addition of* **Buffer PL** *and* **Proteinase K**, *avoid vigorous mixing and pipetting. Mix gently by inverting tube.*
	Nuclease contamination	*Use sterilized glassware, plasticware and wear gloves.* *Ensure that the tissue is completely homogenized in **Buffer PL** and **Proteinase K***
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-PT-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-PT-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.*