GF-1

PLANT DNA EXTRACTION II

USER GUIDE (Version1.0)

Catalog No.
SAMPLE: 5 preps
GF-PT2-050: 50 preps
GF-PT2-100:100 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 Plant DNA Extraction Kit II** is designed for rapid and efficient purification of genomic DNA from various plant samples with high polysaccharides and high phenols content. The purification is based on the usage of denaturing agents to provide efficient plants cells lysis, denaturation of proteins and subsequent release of DNA. Special buffers provided in the kit are optimized to enhance the binding of DNA onto specially-treated glass filter membrane for efficient recovery of highly pure DNA. High-purity genomic DNA is eluted in water or low alt buffers and has a $A_{260/280}$ ratio between 1.8 and 2.0, making it ready to be used in various molecular biology applications.

Kit components

Product Catalog No.	5 Preps SAMPLE	50 Preps GF-PT2-050	100 Preps GF-PT2-100
Components			
GF-1 columns	5	50	100
Collection tubes	5	50	100
Plant Tissue Lysis Buffer **(Buffer PPL)**	2 x 1ml	20ml	40ml
Plant Genomic Binding Buffer (**Buffer PB**)	10ml	2 x 50ml	2 x 100ml
Wash Buffer (concentrate)*	2.4ml	24ml	2 x 24ml
Elution Buffer	1.5ml	10ml	20ml
Proteinase K*	0.11ml	1.05ml	2 x 1.05ml
Handbook	1	1	1

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

The **GF-1 Plant DNA Extraction Kit II** is available as 50 and 100 purifications per kit.

The reagents and materials provided with the kit are for research purposes only.

Note: The **GF-1 Plant DNA Extraction Kit II** is optimized to isolate up to 20µg of DNA from 10-30mg of plant tissue. Plant tissue samples vary in the number of cells depending on age, type of tissue and origin. When processing samples, and do not use more than the recommended starting material as excessive number of cells will overload the column. This would result in reduced yield and purity. We recommend weighing the tissue samples before starting to ensure optimum yield and purity is obtained.

Additional Materials to be Supplied by User

Absolute Ethanol (>95%)
Chloroform
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.

For **SAMPLE (5 preps),**

Add **5.6ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

For **GF-PT2-050 (50 preps),**

Add **56ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

For **GF-PT2-100 (100 preps)**,

Add **56ml** of absolute ethanol into one of the bottles labeled **Wash Buffer**.
Add **56ml** of absolute ethanol into the other bottle labeled **Wash Buffer** only prior to use.

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

Storage and Stability

Store Store 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 PPL** and **Buffer PPB** may exhibit salt precipitation due to cold temperature. If this occurs, simply warm the bottle at 55°C - 65°C with occasional mixing until completely dissolved.

Chemical Hazard

Buffer PPL and **Buffer PPB** contain salts which can be harmful when in contact with skin or swallowed. Always wear gloves and practice standard safety precautions. Do NOT disinfect 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**.

Pre-set waterbath to 65°C.

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

1. **Homogenization**

Cut 100mg 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. If the plant tissue sample cannot be grounded into fine powder, crush the plant tissue samples into mashed form and weigh into tube.

2. **Sample Lysis**

a. Add 280µl of **Buffer PPL** 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.
b. Incubate at 65°C for 10 min 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.
c. Let the tube cool down to room temperature for 1-2 min by gentle vortexing. Add 700µl **Chloroform** into the tube and vortex for another 30 sec. Centrifuge for 5 min at 10,000 x g.

3. **DNA precipitation**

a. Get the tube from centrifuge gently by not disturbing the layer of supernatant. Aliquot 200µl from the very top aqueous supernatant into a new microcentrifuge tube.
Make sure the supernatant that aliquot from the top layer is clear and without any particles.

b. 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.

c. Add 1ml of **Buffer PPB** into the tube and mix well with the 200µl supernatant. Incubate at 65°C for 5 min in a waterbath.

4. 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 4.

5. 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).

6. 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.

7. DNA elution

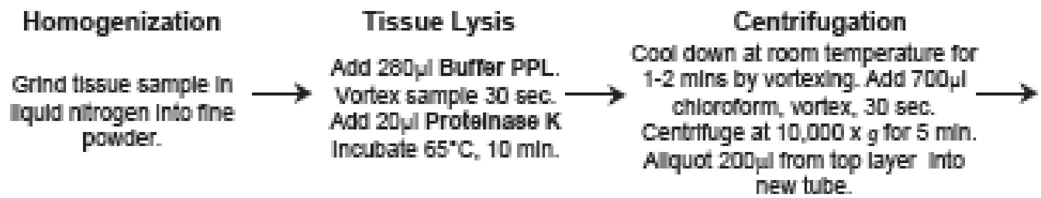
Place the column into a clean microcentrifuge tube. Add 50µl of preheated **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 2 min. Centrifuge at 10,000 x *g* for 1 min to elute DNA. Store DNA at 4°C or -20°C.
Ensure that the Elution Buffer is dispensed directly 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.

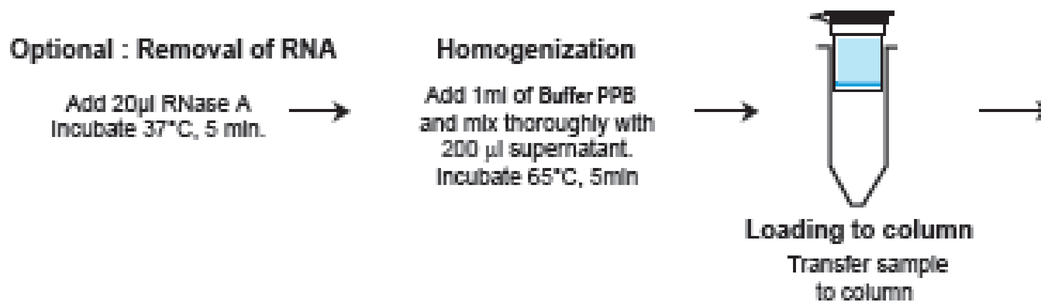
Troubleshooting

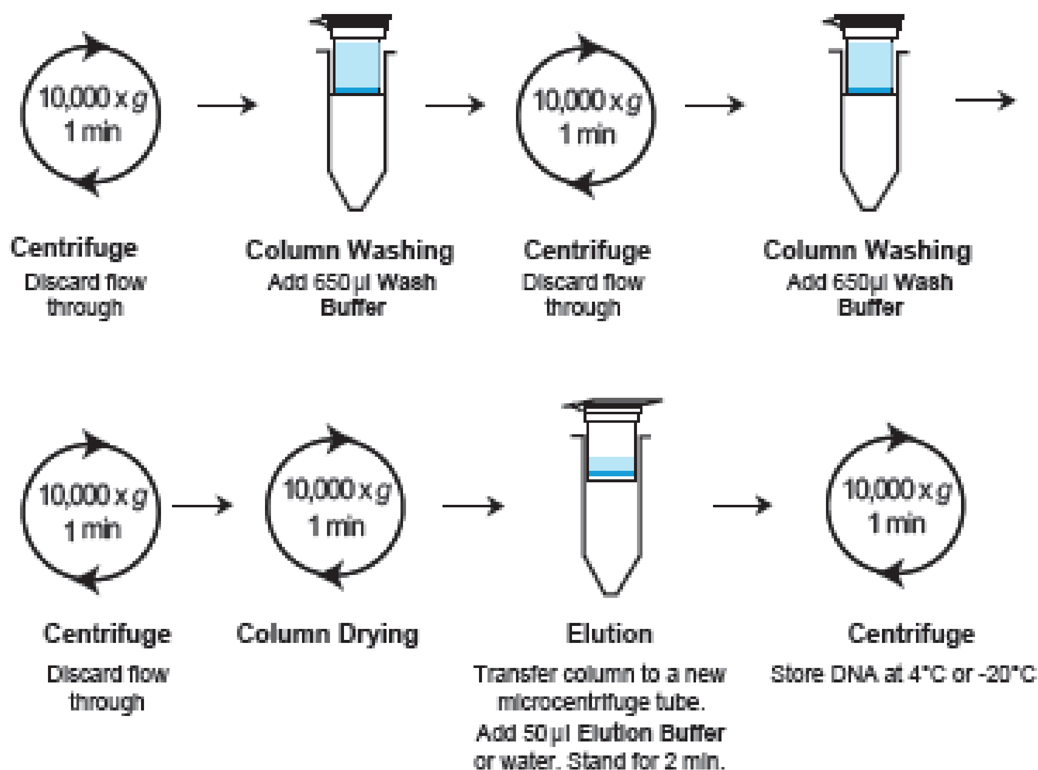
Please note that by not adhering to the recommended protocols, unsatisfactory results related to yield and quality of DNA may occur. If problems arise, please refer to the following:

Problems	Possibility	Suggestions
Low DNA yield	*Sample not thoroughly homogenized*	*Ensure that tissues are completely homogenized in* **Buffer PPL**
	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 PPL*** *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.*
	Column clogged	*Refer to Problems under 'Column clogged'.*
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*

Problems	Possibility	Suggestions
Column clogged	*Overloading of column/ starting material too high*	*Do not use more than 30mg of sample material. If any undigested material remains, spin to remove tissue lysate and transfer supernatant into a new microcentrifuge tube.*
	Sample not thoroughly homogenized	*Vortex sample in* ***Buffer PPL*** *prior t addition of* ***ProteinaseK***
DNA degradation/ smearing	*DNA sheared during purification*	*After the addition of* **Buffer PPL** *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 PPL*** *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.*







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