

Genomic DNA Maxi Kit (Plant)

For research use only

Sample	: 1 g of fresh or 250 mg of dry plant tissue
Yield	: up to 140 µg
Format	: spin column
Operation time	: within 60 minutes

Geneaid



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Introduction

The Genomic DNA Maxi Kit (Plant) provides an efficient method for purifying total DNA (genomic DNA, mitochondrial and chloroplast) from plant tissue and cells. Samples are disrupted by both grinding in liquid nitrogen and lysis buffer incubation. The lysate is treated with RNase A to degrade RNA and then filtered to remove cell debris and salt precipitates. In the presence of the binding buffer, coupled with chaotropic salt, the genomic DNA in the lysate binds to the glass fiber matrix of the spin column (1). Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer or TE. The procedure does not require DNA phenol extraction or alcohol precipitation, and can be completed in less than 1 hour. The purified genomic DNA is ready for use in PCR, Real-time PCR, Southern Blotting and RFLP.

Quality Control

The quality of the Genomic DNA Maxi Kit (Plant) is tested on a lot-to-lot basis by isolating genomic DNA from 250 mg young leaf samples. More than 50 µg of genomic DNA is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	GPM02	GPM10	GPM25
GP1 Buffer	10 ml	50 ml	125 ml
GPX1 Buffer	10 ml	50 ml	125 ml
GP2 Buffer	3 ml	15 ml	30 ml
GP3 Buffer*	8 ml	30 ml	70 ml
(Add Isopropanol)	(16 ml)	(60 ml)	(140 ml)
W1 Buffer	10 ml	45 ml	130 ml
Wash Buffer**	5 ml	25 ml	50 ml
(Add Ethanol)	(20 ml)	(50 ml)	(200 ml)
Elution Buffer	6 ml	30 ml	60 ml
RNase A (10 mg/ml)	100 µl	550 µl	650 µl x 2
Filter Column	2 pcs	10 pcs	25 pcs
GD Maxi Column	2 pcs	10 pcs	25 pcs

Order Information

Product Name	Package Size	Cat. No.
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
96-Well Genomic DNA Kit	2/4/10 x 96 Wells	GBP02/04/10
96-Well Genomic DNA Kit (Plant)	2/4/10 x 96 Wells	GPP02/04/10
Vacuum Manifold (Accessories)	1 set	ZFV04

*Add Isopropanol to the GP3 Buffer prior to initial use (see the bottle label for volume).

**Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

Caution

Some components are irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Genomic DNA Maxi Kit Plant Protocol

Due to various plant species containing different metabolites such as polysaccharides, polyphenols, and proteins, we provide two different lysis buffers. The standard protocol uses GP1 Buffer for lysis of plant samples. For most common plant species, the buffer system ensures purified DNA with high yield and quality. Alternatively, the GPX1 Buffer is provided. The detergent in this lysis buffer is suitable for some plant samples with high polysaccharide content.

- Add Isopropanol to the GP3 Buffer prior to initial use (see the bottle label for volume).
- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: centrifuge tubes

Step 1 Tissue Dissociation	<ul style="list-style-type: none"> ● Cut off 0.5 g (up to 1 g) of fresh or frozen plant tissue or 100 mg (up to 250 mg) of a dried sample. ● Grind the sample under liquid nitrogen to a fine powder and transfer it to a 15 ml centrifuge tube (some plant samples can be disrupted without liquid nitrogen).
Step 2 Lysis	<ul style="list-style-type: none"> ● Add 4 ml of GP1 Buffer (or GPX1 Buffer) and 50 µl of RNase A into the sample tube and mix by vortex. Do not mix GP1 Buffer and RNase A before use. ● Incubate at 65°C for 20 minutes. During incubation, invert the tube every 5 minutes. At this time, preheat the required Elution Buffer (2 ml per sample) to 65°C (for Step 5 DNA Elution). ● Add 1 ml of GP2 Buffer and mix by vortex. ● Incubate on ice for 5 minutes. ● Place a Filter Column in a 50 ml centrifuge tube. ● Transfer the mixture to the Filter Column and centrifuge at 1,000 x g for 5 minutes. ● Discard the Filter Column and carefully transfer the supernatant to a new 50 ml centrifuge tube.
Step 3 DNA Binding	<ul style="list-style-type: none"> ● Add 1.5 volumes of GP3 Buffer (Isopropanol added) to the lysate and vortex immediately for 10 seconds (eg. add 7.5 ml GP3 Buffer to 5 ml of lysate). ● Place a GD Maxi Column in a 50 ml centrifuge tube. ● Transfer the mixture (including any precipitate) to the GD Maxi Column. ● Centrifuge at 4,000 x g for 5 minutes. ● Discard the flow-through and place the GD Maxi Column back in the 50 ml centrifuge tube.
Step 4 Wash	<ul style="list-style-type: none"> ● Add 4 ml of W1 Buffer into the center of the GD Maxi Column. ● Centrifuge at 4,000 x g for 3 minutes. ● Discard the flow-through and place the GD Maxi Column back in the 50 ml centrifuge tube. ● Add 6 ml of Wash Buffer (ethanol added) to the GD Maxi Column. ● Centrifuge at 4,000 x g for 3 minutes. ● Discard the flow-through and place the GD Maxi Column back in the 50 ml centrifuge tube. ● Centrifuge at 4,000 x g for 10 minutes to dry the column matrix. <hr/> <p>Optional Step: Residue Pigment Removal</p> <p>If a few pigments remain on the column matrix, perform this optional step.</p> <ul style="list-style-type: none"> ● Following the Wash Buffer addition, add 4 ml of absolute ethanol to the GD Maxi Column. ● Centrifuge at 4,000 x g for 5 minutes ● Discard the flow-through and place the GD Maxi Column back in the 50 ml centrifuge tube. ● Centrifuge again for 10 minutes at 4,000 x g to dry the column matrix.
Step 5 DNA Elution	<p>Standard elution volume is 1 ml. If less sample is to be used, reduce the elution volume (200-500 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery so the total elution volume is about 2 ml.</p> <ul style="list-style-type: none"> ● Transfer the dried GD Maxi Column to a clean 50 ml centrifuge tube. ● Add 1 ml of preheated Elution Buffer or TE to the center of the column matrix. ● Let stand for 5 minutes or until the Elution Buffer or TE is absorbed by the matrix. ● Centrifuge at 4,000 x g for 3 minutes to elute purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	Too much sample was used. <ul style="list-style-type: none">• Reduce the sample volume or separate it into multiple tubes.
Low Yield	Precipitate was formed at DNA Binding Step <ul style="list-style-type: none">• Reduce the sample material.• Prior to loading the column, break up precipitate in the ethanol-added lysate. Incorrect DNA Elution Step <ul style="list-style-type: none">• Ensure that the Elution Buffer or TE is added to the center of the GD Maxi Column matrix and is absorbed completely.
Eluted DNA does not perform well in downstream applications	Incomplete DNA Elution <ul style="list-style-type: none">• Elute twice to increase yield. Residual ethanol contamination <ul style="list-style-type: none">• Following the Wash Step, dry the GD Maxi Column with additional centrifugation at full speed for 5 minutes or incubate at 60°C for 5 minutes.