For Research Use Only

INSTRUCTION MANUAL

Genomic DNA Mini Kit (Plant)

IB47230

IB47231

IB47232

Sample: up to 100 mg of fresh plant tissue or up to 25 mg of dry plant tissue
Yield: 3-5 µg (100 mg Arabidopsis thaliana leaf), 20-25 µg (100 mg Nicotiana tabacum leaf)
Format: spin column
Time: within 30 minutes
Elution Volume: 30-200 µl
Storage: dry at room temperature (15-25ºC)

Introduction

The Genomic DNA Mini Kit (Plant) provides a quick and easy method for purifying total DNA (including genomic DNA, mitochondrial DNA and chloroplast DNA) from various plant species. Homogenized samples are treated with RNase A then filtered to remove cell debris and salt precipitates. In the presence of the binding buffer, coupled with chaotropic salt, genomic DNA in the lysate binds to the glass fiber matrix of the spin column. Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. The procedure does not require DNA phenol extraction or alcohol precipitation, and can be completed in less than 30 minutes. 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 Mini Kit (Plant) is tested on a lot-to-lot basis according to IBI's ISO-certified quality management system. Genomic DNA is isolated from 50 mg young leaf samples. More than 10 µg of genomic DNA is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Contents

Component	IB47230	IB47231	IB47232
GP1 Buffer	2 ml	50 ml	155 ml
GPX1 Buffer	2 ml	50 ml	100 ml x1 50 ml x1
GP2 Buffer	1 ml	15 µl	40 ml
GP3 Buffer* (Add Isopropanol)	1.5 ml (3 ml)	30 ml (60 ml)	40 ml x2 (80 ml)
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer** (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	30 ml	75 ml
RNase	25 µl	550 µl	550 µl x3
Filter Columns	4	100	300
GD Columns	4	100	300
2 ml Collection Tubes	8	200	600

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

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

Order Information

Product	Package Size	Catalogue #
Genomic DNA Mini Kit (Blood/Cell)	100/300 preps	IB47201/202

Product	Package Size	Catalogue #
Genomic DNA Mini Kit (Tissue)	50/300 preps	IB47221/222
gMax DNA Mini Kit (Blood/Tissue)	100/300 preps	IB47281/282
Genomic DNA Mini Kit (Plant)	100/300 preps	IB47231/232
gSWAB Mini Genomic DNA Kit	100/300 preps	IB47276/277
gBAC Mini DNA Bacteria Kit	100/300 preps	IB47291/292
96 Well Blood gDNA Extraction Kit	4/10 x 96 preps	IB47251/252
gPURE Cell DNA Isolation Kit	100/1000 rxns	IB47431/432

Caution

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

Genomic DNA Mini Kit (Plant) Functional Test Data

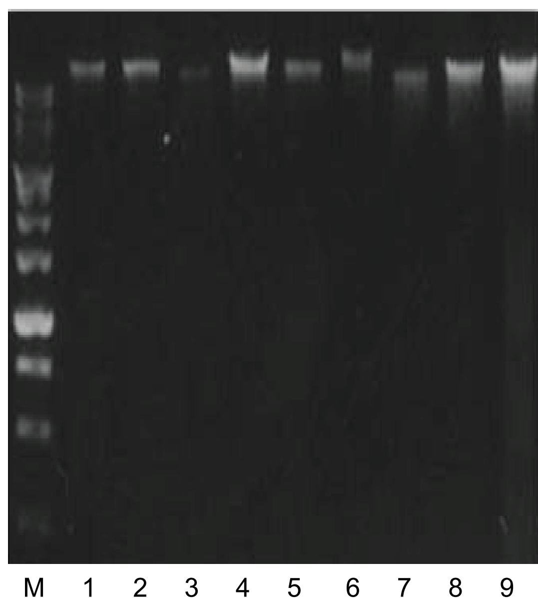

Figure 1. Genomic DNA was extracted from a variety of 100 mg plant species using the Genomic DNA Mini Kit (Plant). A 3 µl aliquot from each 200 µl eluate was analyzed by electrophoresis on a 1% agarose gel.

M = 1 Kb DNA ladder
1. Cinnamomun camphora (Camphor tree)
2. Pisum sativum (Pea sprout)
3. Arabidopsis thaliana (Thale cress)
4. Oryza sativa (Rice)
5. Ipomoea batatas (Sweet potato vine)
6. Rhizoma dioscoreae (Chinese yam)
7. Populus tremula (Aspen)
8. Flammulina velutipes (Mushroom)
9. Oxalis comiculats (Clover)

Genomic DNA Mini Kit (Plant) Protocol

Various plant species contain different metabolites such as polysaccharides, polyphenols, and proteins. The standard protocol uses GP1 Buffer for lysis of most common plant species. Alternatively, GPX1 Buffer is provided with the kit to ensure efficient cell lysis of plant species with high polysaccharide content.

IMPORTANT BEFORE USE!

- Add isopropanol (see the bottle label for volume) to the GP3 Buffer immediately prior to initial use
- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional Requirements: microcentrifuge tubes, isopropanol, absolute ethanol

Step	Procedure
Step 1 Tissue Dissociation	• Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue or 10 mg (up to 25 mg) of dry sample. • Freeze the sample with liquid nitrogen. • Grind the sample to a fine powder then transfer it to a 1.5 ml microcentrifuge tube. NOTE: Some plant samples can be ground sufficiently in the absence of liquid nitrogen.
Step 2 Lysis	NOTE: Mix GP1 Buffer or GPX1 Buffer and RNase A immediately prior to use. • Add 400 μl of GP1 Buffer or GPX1 Buffer and 5 μl of RNase A into the sample tube and mix by vortex. • Incubate at 60oC for 10 minutes. During incubation, invert the tube every 5 minutes. At this time, pre-heat the required Elution Buffer (200 μl per sample) to 60oC (for Step 5 DNA Elution). • Add 100 μl of GP2 Buffer and mix by vortex then incubate on ice for 3 minutes. • Place a Filter Column in a 2 ml Collection Tube then transfer the mixture to the Filter Column. • Centrifuge for 1 minute at 1,000 x g then discard the Filter Column. • Carefully transfer the supernatant from the 2 ml collection tube to a new 1.5 ml microcentrifuge tube.
Step 3 DNA Binding	• Add a 1.5 volume of GP3 Buffer (make sure isopropanol was added) then vortex immediately for 5 seconds. E.g. Add 750 μl of GP3 Buffer to 500 μl of lysate. NOTE: If precipitate appears, break it up as much as possible with a pipette. • Place a GD Column in a 2 ml Collection Tube. • Transfer 700 μl of mixture (and any remaining precipitate) to the GD Column. • Centrifuge at 14-16,000 x g for 2 minutes. • Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. • Add the remaining mixture to the GD Column then centrifuge at 14-16,000 x g for 2 minutes. • Discard the flow-through then place the GD Column back in the 2 ml Collection Tube.
Step 4 Wash	• Add 400 μl of W1 Buffer to the GD Column then centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. • Add 600 μl of Wash Buffer (make sure ethanol was added) to the GD Column. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. • Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix. Optional Residual Pigment Removal Step: If pigments remain on the column, perform this optional step. • Following Wash Buffer addition, add 400 μl of absolute ethanol to the GD Column. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. • Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 5 DNA Elution	Standard elution volume is 100 μl. If less sample is to be used, reduce the elution volume (30-50 μl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to approximately 200 μl. • Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. • Add 100 μl of pre-heated Elution Buffer or TE to the center of the column matrix. • Let stand for 3-5 minutes to ensure the Elution Buffer or TE is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

Troubleshooting

<table>
<tr><th>Problem</th><th>Possible Reasons/Solution</th></tr>
<tr><td>Clogged Column</td><td>Too much sample was used
• Reduce the sample volume or separate it into multiple tubes.</td></tr>
<tr><td>Low Yield</td><td>Precipitate was formed at the DNA Binding step
• Reduce the sample material.
• Following GP3 Buffer addition, break up any precipitate as much as possible prior to loading GD Column.
Incorrect DNA Elution Step
• Ensure that the Elution Buffer or TE is added to the center of the GD Column matrix and is absorbed completely.</td></tr>
<tr><td>Eluted DNA does not perform well in downstream applications</td><td>Incomplete DNA Elution
• Elute twice to increase yield.
Residual ethanol contamination
• Following the Wash Step, dry the GD Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60°C for 5 minutes.</td></tr>
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