HiYield™ 96-Well Genomic DNA Extraction Kit (Plant)

Cat. No.:	YGP96B-2	YGP96B-4	YGP96B-10
Product Name:	HiYield™ 96-Well Genomic DNA Extraction Kit (Plant)		
Reactions:	2	4	10
Format:	96-Well Plates		
Binding Capacity:	Up to 30 μg/well		
Operation:	Centrifuge		
Operation Time:	Within 60 Minutes		

Introduction

HiYield™ 96-Well Genomic DNA Extraction Kit (Plant) provides an efficient method for purifying total DNA (including genomic, mitochondrial and chloroplast DNA) from plant tissue and cells. Samples are disrupted by both grinding in liquid nitrogen and lysis buffer incubation. The procedure does not require DNA phenol extraction or alcohol precipitation and can be completed in 1 hour. The purified genomic DNA is ready for use in PCR, Real-time PCR, Southern Blotting and RFLP.

Features

High-throughput purification of genomic DNA within 1 hour.
Reproducible recoveries for constant results.
Complete removal of all contaminants for reliable downstream applications.

Applications

Purified DNA is ready for direct use in PCR, Southern Blotting, Real-Time PCR, AFLP, RFLP, PADP.

Quality Control

The quality of HiYield™ 96-Well Genomic DNA Extraction Kit (Plant) is tested on a lot-to-lot basis by isolating genomic DNA from a 50 mg young leaf sample. Purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 10 ug. The purified DNA is checked by electrophoresis.

Contents

ITEM	YGP96B-2	YGP96B-4	YGP96B-10
Genomic DNA Binding Plate	2 plates	4 plates	10 plates
350 µl Collection Plate	2 plates	4 plates	10 plates
GP1 Buffer	100 ml	200 ml	500 ml
GPX1 Buffer	100 ml	200 ml	500 ml
GP2 Buffer	25 ml	50 ml	125 ml
GP3 Buffer (concentrated)	150 ml	150 ml	300 ml
W1 Buffer	100 ml	200 ml	500 ml
Wash Buffer (concentrated)	50 ml	50 ml	150 ml
Elution Buffer	30 ml	80 ml	100 ml
RNase A (10mg/ml)	1ml	2ml	5ml

*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

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

Note

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

Protocol

Additional Requirements:

Centrifugation system for 96-well plates, 2 ml collection plates, Isopropanol, absolute ethanol.

Things Before Starting:

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).

Step1 Tissue Dissociation	★Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue or 10 mg (up to 25 mg) of a dried sample. ★Grind the sample under liquid nitrogen to a fine powder and transfer it to each well of a 2 ml collection plate (some plant samples can be disrupted without liquid nitrogen).
Step 2 Lysis	★Add **400 µl of GP1 Buffer** (or **GPX1 Buffer**) and **5 µl of RNase A** into each well of the 2 ml collection plate and mix by pipetting. **Do not mix GP1 Buffer (GPX1 Buffer) and RNase A before use**. ★Incubate at 65ºC for 10 minutes. During incubation, gently shake the plate every 5 minutes. At this time, preheat the required **Elution Buffer** (200 µl per sample) to 65ºC (for Step 5 DNA Elution). ★Add **100 µl of GP2 Buffer** to each well of the 2 ml collection plate and mix by pipetting. ★Incubate at 4ºC for 3 minutes. ★Centrifuge at 1,000 x g for 10 minutes. ★Transfer the supernatant to a new 2 ml collection plate.

Step 3 DNA Binding	★Add a 1.5 volume of **GP3 Buffer** (Isopropanol added) to the lysate and gently shake immediately for 5 seconds (eg. add 750 µl GP3 Buffer to 500 µl lysate). ★Place a **Genomic DNA Binding Plate** on a new 2 ml collection plate. ★Transfer 700 µl of the mixture to each well of a **Genomic DNA Binding Plate.** ★Centrifuge at 1,000 x g for 2 minutes. ★Discard the flow-through from the 2 ml collection plate. Place the **Genomic DNA Binding Plate** back on the 2 ml collection plate and transfer the remaining mixture to each well of the **Genomic DNA Binding Plate**. ★Centrifuge again at 1,000 x g for 2 minutes. ★Discard the flow-through and place the **Genomic DNA Binding Plate** back on the 2 ml collection plate.
Step 4 Wash	★Add **400 µl of W1 Buffer** to each well of the **Genomic DNA Binding Plate**. ★Centrifuge at 1,000 x g for 30 seconds.. ★Discard the flow-through and place the **Genomic DNA Binding Plate** back on the 2 ml collection plate. ★Add **600 µl of Wash Buffer** (ethanol added) to each well of the **Genomic DNA Binding Plate**. ★Centrifuge at 1,000 x g for 30 seconds. ★Discard the flow-through and place the **Genomic DNA Binding Plate** back on the 2 ml collection plate. ★Centrifuge again for 3 minutes at 1,000 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 **Genomic DNA Binding Plate** to a **350 ul Collection Plate**. ★Add **100 µl of preheated Elution Buffer** or TE to the center of each well of the **Genomic DNA Binding Plate**. ★Let stand for 3-5 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ★Centrifuge at 1,000 x g for 1 minute to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	**Too much sample was used** →Reduce the sample volume.
Low Yield	**Precipitate was formed at DNA Binding Step** →Reduce the sample material. →Prior to loading the plate, break up the precipitate in the ethanol-added lysate. **Incorrect DNA Elution Step** →Ensure that the Elution Buffer or TE is added to the center of each well of the Genomic DNA Binding Plate and is absorbed completely. →Ensure to preheat the Elution Buffer or TE prior to adding to the Genomic DNA Binding Plate.
Eluted DNA does not perform well in downstream applications	**Incomplete DNA Elution** →Elute twice to increase yield. **Residual ethanol contamination** →Following the Wash Step, dry the plate with additional vacuum or incubate at 60ºC for 5 minutes.