Clean Plant DNA Kit

Catalog Nos. CP-D0096, CP-D0384
Manual revision v1.01

Contents

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Introduction and Principle

The Clean Plant DNA Kit allows rapid and reliable isolation of high-quality genomic DNA from a wide variety of plant species and tissues. Up to ninety-six 50 mg samples of wet tissue (or 15 mg dry tissue) can be processed in less than one hour. The system combines CleanNA's buffer chemistry with the convenience of CleanNA's PRG Beads to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. This kit is designed for manual or fully automated high throughput preparation of genomic, chloroplast, and mitochondrial DNA. Purified DNA is suitable for PCR, restriction digestion, and hybridization applications. There are no organic extractions thereby reducing plastic waste and decreasing hands-on time to allow multiple samples to be processed in parallel.

The method will produce genomic DNA suitable for PCR and qPCR. Because of the high throughput processing method, high molecular weight genomic DNA may be sheared during lysis and may not be suitable for certain hybridization-based assays or Southern blotting.

Plant samples are disrupted in a homogenizer/bead based milling equipment. PGL1 Buffer is added to lyse the sample. Supernatant is then transferred to a new processing plate where PRG Beads are added to bind to the DNA. Following a few wash steps, DNA is eluted from the PRG Beads for downstream application.

Kit Contents and Materials

Kit Contents:

Product Number	CP-D0096	CP-D0384	Storage
Preparations	1 x 96	4 x 96	
PRG Beads	1.7 mL	7 mL	2-8ºC
PGL1 Buffer	60 mL	240 mL	15-25ºC
PGW1 Buffer	39 mL	143 mL	15-25ºC
PGW2 Buffer	12 mL	44 mL	15-25ºC
PGW3 Wash Buffer	36 mL	144 mL	15-25ºC
Elution Buffer	15 mL	60 mL	15-25ºC
RNase A	1.5 mL	3.2 mL	2-8ºC

Materials and Reagents to be supplied by User:

- Centrifuge capable of at least 3,000-5,000 x g
- Rotor adapter for 96-well deep-well plates
- Magnetic separation device for 96-well deep-well plates
- 96-well deep-well plates compatible with magnetic separation device
- Incubators capable of 56°C and 65°C
- Equipment for disrupting plant tissue (Geno/Grinder 2010 or MM300 Mixer Mill and tungsten carbide beads)
- 8- or 12-channel pipette
- Reagent reservoir
- Sealing film
- Sealed deep-well plate or capped microtube rack for sample disruption
- 100% ethanol
- Isopropanol

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Preparation of Reagents

Dilute PGW1 Buffer with 100% ethanol as follows and store at room temperature.

PGW1 Buffer	100% Ethanol to be Added
CP-D0096	21 mL
CP-D0384	77 mL

Dilute PGW2 Buffer with isopropanol as follows and store at room temperature.

PGW2 Buffer	Isopropanol to be Added
CP-D0096	48 mL
CP-D0384	176 mL

Dilute PGW3 Wash Buffer with 100% ethanol as follows and store at room temperature.

PGW3 Wash Buffer	100% Ethanol to be Added
CP-D0096	84 mL
CP-D0384	336 mL

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Clean Plant DNA Kit
DNA Isolation from Fresh or Frozen Specimens

The following method can be used for faster processing of samples. For fresh samples, this protocol may result in shearing of DNA. For frozen samples, this protocol may result in lower yields and sheared DNA. Purified DNA is suitable for PCR and qPCR.

Before Starting:
Prepare PGW1 Buffer, CSW2 Buffer, and PGW3 Wash Buffer according to the instructions in the Preparing Reagents section on Page 3
Set an incubator to 56°C
Heat Elution Buffer to 65°C

Protocol:

1. Grind 30–50 mg plant sample using a mechanical grinder such as Geno/Grinder.

   

   Note: To prepare samples in 96-well plate format, place samples in a sealed 96-well deep-well plate or capped microtube rack in the presence of one or two grinding beads. Process in the MM300 Mixture Mill or Geno/Grinder Mixture Mill following the manufacturer's instructions.

2. Add 500 µL PGL1 Buffer to each well. Vortex to mix thoroughly.
3. Incubate at 56°C for 30 minutes.
4. Centrifuge at 4,000 x *g* for 10 minutes.
5. Carefully transfer 400 µL cleared lysate to a new 96-well deep-well plate, making sure not to disturb the pellet or transfer any debris.k

   

   Note: It is critical to leave the pellet undisturbed and avoid transferring debris as these can reduce yield..

6. Add 5 µL RNase A. Vortex to mix thoroughly.
7. Incubate at room temperature for 10 minutes.
8. Thoroughly resuspend the PRG beads by pipetting or vortexing.
9. Add 400 µL isopropanol and 15 µL PRG Beads. Vortex to mix thoroughly.
10. Incubate at room temperature for 5 minutes.
11. Place the plate on a magnetic separation device to magnetize the PRG Beads. Incubate at room temperature until the PRG Beads are completely cleared from solution.
12. Aspirate and discard the cleared supernatant. Do not disturb the PRG Beads.
13. Remove the plate from the magnetic separation device.
14. Add 500 µL PGW1 Buffer. Vortex briefly or pipet up and down to resuspend the PRG Beads.

    

    Note: PGW1 Buffer must be diluted with 100% ethanol prior to use. Please see Page 3 for instructions.

15. Place the plate on a magnetic separation device to magnetize the PRG Beads. Incubate at room temperature until the PRG Beads are completely cleared from solution.
16. Aspirate and discard the cleared supernatant. Do not disturb the PRG Beads.
17. Remove the plate from the magnetic separation device.

18. Add 500 µL PGW2 Buffer. Vortex briefly or pipet up and down to resuspend the PRG Beads.

Note: PGW2 Buffer must be diluted with isopropanol prior to use. Please see Page 3 for instructions.

19. Place the plate on a magnetic separation device to magnetize the PRG Beads. Incubate at room temperature until the PRG Beads are completely cleared from solution.

20. Aspirate and discard the cleared supernatant. Do not disturb the PRG Beads.

21. Remove the plate from the magnetic separation device.

22. Add 500 µL PGW3 Wash Buffer. Vortex briefly or pipet up and down to resuspend the PRG Beads.

Note: PGW3 Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 3 for instructions.

23. Place the plate on a magnetic separation device to magnetize the PRG Beads. Incubate at room temperature until the PRG Beads are completely cleared from solution.

24. Aspirate and discard the cleared supernatant. Do not disturb the PRG Beads.

25. Repeat Steps 20-23 for a second PGW3 Wash Buffer wash step.

26. Leave the plate on the magnetic separation device for 10 minutes to air dry the magnetic particles. Remove any Residual liquid with a pipettor.

27. Remove the plate from the magnetic separation device.

28. Add 100 µL Elution Buffer heated to 65°C. Vortex briefly or pipet up and down to resuspend the PRG Beads.

29. Incubate at 65°C for 10 minutes.

30. Place the plate on a magnetic separation device to magnetize the PRG Beads. Incubate at room temperature until the PRG Beads are completely cleared from solution.

31. Transfer the supernatant containing the eluted DNA to a clean 96-well microplate (not supplied).

32. Store DNA at -20°C.

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Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact your local distributor.

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA yield	Incomplete disruption of starting material	For both fresh and frozen samples, make sure to grind samples completely.
	Poor lysis of tissue	Decrease amount of starting material.
	DNA lost during wash	Dilute PGW3 Wash Buffer by adding appropriate volume of ethanol prior to use (Page 3).
Problems in downstream applications	Salt carryover	PGW3 Wash Buffer must be at room temperature.
	Ethanol carryover	Dry the PRG Beads completely before adding elution buffer.

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Plant DNA Kit (1 x 96)	CP-D0096
Clean Plant DNA Kit (4 x 96)	CP-D0384

Notes

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