Clean Plant PK DNA Kit

Catalog Nos. CPPK-D0096, CPPK-D0384
Manual revision v2.00

Contents

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Introduction and Principle

The Clean Plant PK DNA Kit allows rapid and reliable isolation of high-quality genomic DNA from a wide variety of plant samples. The optimized buffer chemistry, including a Proteinase K treatment, allows the isolation of genomic DNA also from difficult plant species and tissues. The lysis and binding buffers are specifically designed to minimize co-purification of polysaccharides and polyphenols.

Our Clean Plant PK DNA Kit combines our propriety buffer system with the convenience of our CleanNA Particles 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, Next Generation Sequencing, 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.

Plant samples are disrupted in a homogenizer/bead based milling equipment CPPK Lysis buffer and Proteinase K are added to lyse the sample including the more difficult plant cell walls. Supernatant is then transferred to a new processing plate where CleanNA Particles CPPK 1 are added to bind the DNA. Following a few wash steps, DNA is eluted from the CPPK 1 beads.

For samples which show coloration and/or a viscous eluate after isolation, an optional purification step can be performed. CleanNA Particles CPPK 2 and binding buffer are added to the eluted DNA, allowing re-binding of the genomic DNA. Following a few ethanol wash steps, DNA is eluted from the CPPK 2 beads ready for downstream applications.

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Kit Contents and Materials

Kit Contents:

Product	CPPK-D0096	CPPK-D0384	Storage
Preps	1 x 96	4 x 96	
CPPK Lysis Buffer	80 mL	2 x 150 mL	15-25^{0}C
CPPK Wash Buffer 1	39 mL	143 mL	15-25^{0}C
CPPK Wash Buffer 2	12 mL	44 mL	15-25^{0}C
Elution Buffer	60 mL	240 mL	15-25^{0}C
CleanNA Particles CPPK 1	2.2 mL	9 mL	2-8^{0}C
CleanNA Particles CPPK 2	2.2 mL	9 mL	2-8^{0}C
Rnase A	1.5 mL	3.2 mL	2-8^{0}C
CPPK Wash Buffer 3	36 mL	2 x 75 mL	15-25^{0}C
Binding Buffer CPPK	60 mL	240 mL	15-25^{0}C
CPPK Rebind Buffer	50 mL	200 mL	15-25^{0}C
Proteinase K Solution	2.2 mL	8.8 mL	15-25^{0}C

Materials and Reagents to be supplied by User:

- Equipment for disrupting plant tissue (Geno/Grinder 2010 or MM300 Mixer Mill and tungsten carbide beads)
- Incubators capable of 56°C and 65°C
- 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 (Recommended Clean Magnet Plate 96-Well RN50 (Part# CMAG-RN50))
- 96-well deep-well plates compatible with magnetic separation device
- Vortexer
- 8- or 12-channel pipette
- Reagent reservoir
- Sealing film
- Sealed deep-well plate or capped microtube rack for sample disruption
- 100% ethanol
- 100% isopropanol
- Optional: 85% ethanol
- Optional: nuclease-free water

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Preparation of Reagents

Dilute CPPK Wash Buffer 1 with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
CPPK-D0096	21 mL
CPPK-D0384	77 mL

Prepare CPPK Wash Buffer 2 with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
CPPK-D0096	48 mL
CPPK-D0384	176 mL

Prepare CPPK Wash Buffer 3 with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
CPPK-D0096	84 mL
CPPK-D0384	175 mL

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Clean Plant PK DNA Kit
Protocol for DNA Isolation from Fresh or Frozen Specimens

Before Starting:

- Prepare CPPK Wash Buffer 1, CPPK Wash Buffer 2, and CPPK Wash Buffer 3 according to the instructions in the Preparing Reagents section on Page 4.
- 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 700 µL CPPK Lysis Buffer and 20 µL Proteinase K Solution 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 500 µL cleared lysate to a new 96-well deep-well plate, making sure not to disturb the pellet or transfer any debris.

   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 2 minutes.

8. Add 500 µL Binding Buffer CPPK and 20 µL CleanNA Particles CPPK 1. Vortex to mix thoroughly.

9. Incubate at room temperature for 5 minutes. Vortex briefly every 90 seconds to resuspend magnetic beads.

   Note: If using a liquid handler with orbital shaker, continue to shake for the entire duration of this step.

10. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CPPK 1. Incubate at room temperature until the CleanNA Particles CPPK 1 are completely cleared from solution.

11. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPPK 1.

12. Remove the plate from the magnetic separation device.

13. Add 500 µL CPPK Wash Buffer 1. Vortex briefly or pipet up and down to resuspend the CleanNA Particles CPPK 1.

    Note: CPPK Wash Buffer 1 Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

14. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CPPK 1. Incubate at room temperature until the CleanNA Particles CPPK 1 are completely cleared from solution.

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15. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPPK 1.

16. Remove the plate from the magnetic separation device.

17. Add 500 µL CPPK Wash Buffer 2. Vortex briefly or pipet up and down to resuspend the CleanNA Particles CPPK 1.

Note: CPPK Wash Buffer 2 Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions.

18. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CPPK 1. Incubate at room temperature until the CleanNA Particles CPPK 1 are completely cleared from solution.

19. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPPK 1.

20. Remove the plate from the magnetic separation device.

21. Add 500 µL CPPK Wash Buffer 3. Vortex briefly or pipet up and down to resuspend the CleanNA Particles CPPK 1.

Note: CPPK Wash Buffer 3 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

22. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CPPK 1. Incubate at room temperature until the CleanNA Particles CPPK 1 are completely cleared from solution.

23. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPPK 1.

24. Repeat Steps 20-23 for a second CPPK Wash Buffer 3 wash step.

25. Leave the plate on the magnetic separation device for 10 minutes to air dry the CleanNA Particles CPPK 1. Remove any residue liquid with a pipettor.

Alternative Ethanol Removal Step: Instead of performing Step 25, complete the step below.

- With the plate on the magnetic separation device, add 500 µL nuclease-free water and immediately aspirate (within 60 seconds).
- Continue to Step 26 below.

26. Remove the plate from the magnetic separation device.

27. Add 100-200 µL Elution Buffer heated to 65°C. Vortex briefly or pipet up and down to resuspend the CleanNA Particles CPPK 1.

28. Incubate at 65°C for 5 minutes.

29. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CPPK 1. Incubate at room temperature until the CleanNA Particles CPPK 1 are completely cleared from solution.

30. Transfer the supernatant containing the eluted DNA to a clean 96-well microplate and store at -20°C.

Note: If performing the secondary purification protocol (continue to next page), transfer the supernatant to a new 96-well plate with a capacity of 800 µL.

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Optional Secondary Purification Protocol

The following steps can be performed if the samples contain any coloration or Elution Buffer is found to be viscous during pipetting. Performing an additional purification can help improve downstream performance.

31. Add 400 µL CPPK Rebind Buffer and 20µL CleanNA Particles CPPK 2 to the sample. Vortex briefly or pipet up and down to resuspend the CleanNA Particles CPPK 2.

32. Incubate at room temperature for 3 minutes.

33. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CPPK 2. Incubate at room temperature until the CleanNA Particles CPPK 2 are completely cleared from solution.

34. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPPK 2.

35. Leave the plate on the magnetic separation device.

36. Add 400 µL 85% ethanol.

37. Incubate at room temperature for 30 seconds.

38. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPPK 2.

39. Repeat Steps 35-38 for a second ethanol wash step.

40. Leave the plate on the magnetic separation device for 10 minutes to air dry the CleanNA Particles CPPK 2. Remove any residue liquid with a pipettor.

Alternative Ethanol Removal: Instead of performing Step 40, complete the step below.

- With the plate on the magnetic separation device, add 400 µL nuclease-free water and immediately aspirate (within 60 seconds).
- Continue to Step 41.

41. Add 50-200 µL Elution Buffer heated to 65°C. Vortex briefly or pipet up and down to resuspend the CleanNA Particles CPPK 2.

42. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CPPK 2. Incubate at room temperature until the CleanNA Particles CPPK 2 are completely cleared from solution.

43. Transfer the supernatant containing the eluted DNA to a clean 96-well microplate.

44. Store 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.
		Increase lysis time to overnight.
	DNA lost during wash	Dilute CPPK Wash Buffer 3 by adding appropriate volume of ethanol prior to use (Page 4).
		If performing water wash step, ensure to remove the water within 60 seconds
		If drying by air, leave the plate on the magnetic separation device during drying
Problems in downstream applications	Salt carryover	CPPK Wash Buffer 3 must be at room temperature.
	Ethanol carryover	Ensure to perform the water “wash” to remove final traces of ethanol
		Dry the CleanNA Particles CPPK 1 and/or CleanNA Particles CPPK 2 completely before adding elution buffer.
		Perform Water Wash step instead of drying magnetic beads

Ordering Information

Contact your local distributor to order.

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

Product	Part Number
Clean Magnet Plate 96-Well RN50	CMAG-RN50

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