Clean Plant PK DNA Kit

USER MANUAL

Manual revision v5.00

Catalog Numbers:
CPPK-D0096: 96 preps
CPPK-D0384: 384 preps

Batch No: See package

Shipping: room temperature

Storage and stability: CleanNA Particles CPPK 1 and Rnase A should be stored at 4°C upon receipt, store all other components at room temperature. See page 3 for more storage information.

Intended use: Clean plant PK DNA Kit is intended for use by professional users trained in molecular biology techniques. It is designed to use manually or on a liquid handling workstation for molecular biology applications.

Quality Control: Each lot of Clean Plant PK is tested against predetermined specifications to ensure consistent product quality. If in any case inconsistencies occur, please contact us at info@cleanna.com or +31 (0) 182 22 33 50.

Safety precautions: When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Please refer to the material safety data sheet for further information.

Emergency: In case of a medical emergency due to the use of this product, contact your local poison control center. When a severe incident occurs, please inform CleanNA at +31 (0) 182 22 33 50 or info@cleanna.com.

Expiry: When stored under the recommended conditions and handled correctly, full activity is retained until the expiry date on the outer box label.

FOR RESEARCH USE ONLY

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Introduction and Principle

The Clean Plant PK DNA Kit allows for the 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 DNA also from difficult plant species and tissues.

Our Clean Plant PK DNA Kit combines our propriety buffer system with the convenience of our CleanNA Particles CCPK 1. The lysis and binding buffers are specifically designed to minimize co-purification of polysaccharides, polyphenols and other enzyme inhibitors from plant tissue lysates. This kit can be used manually, or automated on laboratory liquid handlers (e.g. Dynamic Devices LYNX™, Hamilton STAR™) for high throughput preparation of genomic, chloroplast and mitochondrial DNA.

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 particles.

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.

Kit Contents and Materials

Kit Contents:

Product	CPPK-D0096	CPPK-D0384	Storage
Preps	1 x 96	4 x 96	n/a
CPPK Lysis Buffer	80 mL	2 x 150 mL	15-25°C
CPPK Wash Buffer 1	39 mL	143 mL	15-25°C
CPPK Wash Buffer 2	12 mL	44 mL	15-25°C
Elution Buffer	60 mL	240 mL	15-25°C
CleanNA Particles CPPK 1	2.2 mL	9 mL	2-8°C
RNase A (25 mg/mL)	1.5 mL	3.2 mL	2-8°C
CPPK Wash Buffer 3	36 mL	2 x 75 mL	15-25°C
Binding Buffer CPPK	60 mL	240 mL	15-25°C
Proteinase K Solution	2.2 mL	8.8 mL	15-25°C (for storage > 6 months, store at 2-8°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: molecular biology grade water

Preparation of Reagents

CPPK Wash Buffer 1
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

CPPK Wash Buffer 2
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

CPPK Wash Buffer 3
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

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 particles.

   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.
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 molecular biology grade 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.

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 completely before adding elution buffer.
		Perform water wash step instead of drying magnetic particles.

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	CMAG-96-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
5	27/FEB/2024	Protocol for DNA Isolation from Fresh or Frozen Specimens	Deleted the Optional Secondary Purification Protocol
4.00	October 2021	Total revision	Language and layout modifications
		Kit contents	Included storage conditions for long term storage of Proteinase K solution
3.00	September 2020	Total revision	New lay-out
			Important information added at page 1 (before contents)