Clean Plant PK DNA Kit

Instructions for Use

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For Research Use Only

REF **CPPK-D0096, CPPK-D0384**

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Contents

Intended Use

The 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.

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 HB. 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 our CleanXtract 96 or liquid handlers (e.g. Dynamic Devices LYNX™, Hamilton STAR™) for 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.

Schematic Overview

Disrupted plant samples are lysed with the optimized lysis buffer and Proteinase K. After the addition of RNase A, CleanNA particles HB are added and the DNA attaches to their surface. The CleanNA magnetic particles are separated from the lysate by a magnetic separation device. Following a few wash steps to remove trace contaminants, the purified DNA is eluted from the CleanNA particles HB using an elution buffer or molecular biology grade water. The eluted DNA is directly suitable for downstream applications.

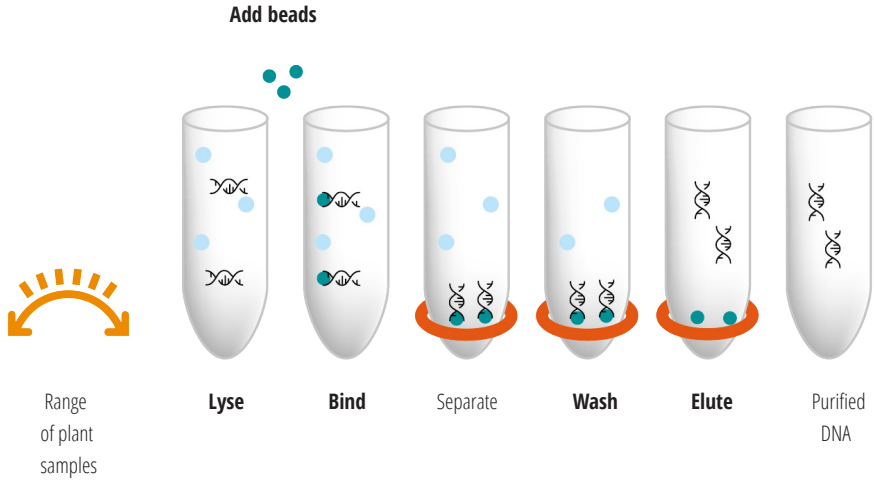

Figure 1: Schematic overview of the Clean Plant PK DNA Kit extraction procedure.

Materials Provided

Kit Contents:

Component	CPPK-D0096 (1x96 preps)	CPPK-D0384 (384 preps)
PL Lysis	80 mL	2 x 150 mL
BA Binding	60 mL	240 mL
PWB Wash 01	39 mL	143 mL
PWB Wash 02	12 mL	44 mL
EX Wash	36 mL	2 x 75 mL
Elution Buffer	60 mL	250 mL
CleanNA Particles HB	2,2 mL	9 mL
RNase A (10 mg/mL)	1.5 mL	3.2 mL
Proteinase K (20 mg/mL)	2.2 mL	9 mL

Reagent Shipping, Storage and Handling

Clean Plant PK DNA Kit is shipped at room temperature (15-25 °C). Do not freeze the components of the Clean Plant PK DNA Kit. After the components have been frozen, the kit is no longer suitable for use. Do not use the Clean Plant PK DNA Kit after the expiration date stated on the outer box label.

Component	Storage Temperature
PL Lysis	15-25 °C
BA Binding	15-25 °C
PWB Wash 01	15-25 °C
PWB Wash 02	15-25 °C
EX Wash	15-25 °C
Elution Buffer	15-25 °C
CleanNA Particles HB	2-8 °C
RNase A (10 mg/mL)	2-8 °C
Proteinase K (20 mg/mL)	15-25 °C (for storage > 12 months, store at 2-8 °C)

⚠ **Note:** Check all buffers for precipitates prior to usage. Any precipitates can be re-dissolved by warming the buffer(s) to 37ºC and shaking gently.

Warnings

Read the instructions carefully before using the kit.

Do not mix several kit LOT numbers.

The LOT number on the CleanNA Particles HB and RNase A box packaging is different from the LOT number on the CleanNA Particles HB or RNase A bottle. The LOT number on the box matches the LOT number of the whole kit and the one on the bottles is specifically for the particles/RNase A. Since the CleanNA Particles HB and RNase A are stored at a different temperature than the rest of the kit, please make sure that the LOT number on the box packaging of the particles matches the LOT number of the kit before use.

Precautions

When working with chemicals, always follow your facility's procedures and universal precautions by using disposable gloves, safety glasses, a labcoat etc.

For all safety information, please consult the safety data sheet (SDS).

PL Lysis	
	Causes skin irritation. Causes serious eye irritation. May cause an allergic skin reaction. Harmful to aquatic life with long lasting effects. **IF ON SKIN**: Wash with plenty of water and soap. **IF IN EYES**: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If skin irritation or rash occurs: Get medical advice/attention. If eye irritation persists: Get medical advice/attention. Take off contaminated clothing and wash it before reuse.
BA Buffer	
	Toxic to aquatic life with long lasting effects. Causes serious eye damage. Harmful if swallowed. Causes skin irritation. Contact with acids liberates very toxic gas. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor/physician/first aider. Collect spillage. **IF SWALLOWED:** Rinse mouth. Call a POISON CENTER/doctor/physician/first aider if you feel unwell. **IF ON SKIN:** Wash with plenty of water and soap. If skin irritation occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse.
PWB Wash 01	
	May cause fire or explosion; strong oxidiser. Harmful if swallowed. In case of fire: Use water spray/fog to extinguish. In case of major fire and large quantities: Evacuate area. Fight fire remotely due to the risk of explosion. **IF ON CLOTHING:** Rinse immediately contaminated clothing and skin with plenty of water before removing clothes. **IF SWALLOWED:** Call a POISON CENTER/doctor/physician/first aider if you feel unwell. Rinse mouth.

PWB Wash 02	
 	May cause fire or explosion; strong oxidiser. Harmful if swallowed. In case of fire: Use water spray/fog to extinguish. In case of major fire and large quantities: Evacuate area. Fight fire remotely due to the risk of explosion. **IF ON CLOTHING:** Rinse immediately contaminated clothing and skin with plenty of water before removing clothes. **IF SWALLOWED:** Call a POISON CENTER/doctor/physician/first aider if you feel unwell. Rinse mouth.
Proteinase K (20 mg/mL)	
	May cause allergy or asthma symptoms or breathing difficulties if inhaled. **IF INHALED:** Remove person to fresh air and keep comfortable for breathing. If experiencing respiratory symptoms: Call a POISON CENTER/doctor/physician/first aider.
RNase A (10 mg/mL)	
	May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause an allergic skin reaction. **IF INHALED:** Remove person to fresh air and keep comfortable for breathing. If experiencing respiratory symptoms: Call a POISON CENTER/doctor/physician/first aider. **IF ON SKIN:** Wash with plenty of water and soap. If skin irritation or rash occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse.

Note: For safe disposal, please consult your local waste regulations.

Quality Control

CleanNA produces each lot of Clean Plant PK DNA Kit according to predetermined and validated protocols in the Quality Management System (QMS). CleanNA's QMS is EN-ISO 13485 certified.

Materials and Equipment to be Supplied by User

Materials and reagents to be supplied by user for the Protocol for DNA Isolation from Fresh or Frozen Specimens:

- 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-96-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
- Ethanol absolute
- Isopropanol absolute
- Optional: molecular biology grade water

Preparation of Reagents

PWB Wash 01

Prepare PWB Wash 01 with absolute ethanol as follows and store at room temperature.

Kit	Absolute ethanol to be added
CPPK-D0096	21 mL
CPPK-D0384	77 mL

PWB Wash 02

Dilute PWB Wash 02 with absolute isopropanol as follows and store at room temperature.

Kit	Absolute isopropanol to be added
CPPK-D0096	48 mL
CPPK-D0384	176 mL

EX Wash

Prepare PWB Wash 01 with absolute ethanol as follows and store at room temperature.

Kit	Absolute ethanol to be added
CPPK-D0096	84 mL
CPPK-D0384	175 mL

Protocol for DNA isolation from fresh or frozen specimens

Before Starting:

• Prepare PWB Wash 01, PWB Wash 02, and EX Wash according to the instructions in the Preparing Reagents section on Page 11.
• 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 Greno/Grinder Mixture Mill following the manufacturer's instructions.

2. Add 700 µL PL Lysis and 20 µL Proteinase K 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 BA Binding and 20 µL CleanNA Particles HB. 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 HB. Incubate at room temperature until the CleanNA Particles HB are completely cleared from solution.
11. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles HB.
12. Remove the plate from the magnetic separation device.
13. Add 500 µL PWB Wash 01. Vortex briefly or pipet up and down to resuspend the CleanNA Particles HB.

⚠ **Note:** PWB Wash 01 must be diluted with ethanol absolute prior to use. Please see Page 11 for instructions.

14. Place the plate on a magnetic separation device to magnetize the CleanNA Particles HB. Incubate at room temperature until the CleanNA Particles HB are completely cleared from solution.
15. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles HB.
16. Remove the plate from the magnetic separation device.
17. Add 500 µL PWB Wash 02. Vortex briefly or pipet up and down to resuspend the CleanNA Particles HB.

⚠ **Note:** PWB Wash 02 must be diluted with isopropanol prior to use. Please see Page 11 for instructions.

18. Place the plate on a magnetic separation device to magnetize the CleanNA Particles HB. Incubate at room temperature until the CleanNA Particles HB are completely cleared from solution.
19. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles HB.
20. Remove the plate from the magnetic separation device.
21. Add 500 µL EX Wash. Vortex briefly or pipet up and down to resuspend the CleanNA Particles HB.

⚠ **Note:** EX Wash must be diluted with ethanol absolute prior to use. Please see Page 11 for instructions.

22. Place the plate on a magnetic separation device to magnetize the CleanNA Particles HB. Incubate at room temperature until the CleanNA Particles HB are completely cleared from solution.
23. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles HB.
24. Repeat Steps 20-23 for a second EX Wash step.
25. Leave the plate on the magnetic separation device for 10 minutes to air dry the CleanNA Particles HB. 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 HB.

28. Incubate at 65°C for 5 minutes.
29. Place the plate on a magnetic separation device to magnetize the CleanNA Particles HB. Incubate at room temperature until the CleanNA Particles HB are completely cleared from solution.
30. Transfer the supernatant containing the eluted DNA to a clean 96-well microplate and store at -20°C.

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	Suggestion
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 EX Wash by adding the appropriate volume of ethanol prior to use.
		If performing the 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 down-stream applications	Salt carryover	EX Wash must be at room temperature.
	Ethanol carryover	Ensure to perform the water "wash" to remove final traces of ethanol.
		Dry the CleanNA Particles HB completely before adding elution buffer.
		Perform the water wash step instead of drying magnetic particles.

Symbols

	Reference number
	Manufacturer
	Caution
	Temperature limit
	Expiration date
	Lot number

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Plant PK DNA Kit 96 preps	CPPK-D0096
Clean Plant PK DNA Kit 384 preps	CPPK-D0384

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

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
6	10/JUN/2024	Total revision	Change of buffer names
			New lay-out according to the CleanNA corporate style.
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)

Notes

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