PLANT DNA PURIFICATION KIT

User Manual

26-022.....5 preps
26-023.....50 preps
26-023B.....50 preps
P/N 03-293-10 REV. B

OMNI International
The Homogenizer Company®

INTRODUCTION

The Omni Plant DNA Purification Kit is designed for efficient recovery of genomic DNA up to 40 kb in size from fresh, frozen, or dried plant tissue samples rich in polysaccharides or having a lower DNA content. Up to 100 mg wet tissue (or 30 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the Omni Mini DNA Column matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization applications.

OVERVIEW

If using the Omni Plant DNA Purification Kit for the first time, please read this booklet to become familiar with the procedures. This procedure relies on the well-established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding of the Omni Mini DNA Column matrix, to isolate high-quality DNA. Samples are homogenized and lysed in a high salt buffer containing CTAB and extracted with chloroform to remove polysaccharides and other components that interfere with many routine DNA isolations and downstream applications. Binding conditions are adjusted, and DNA is purified using an Omni DNA Mini Column. Salts, proteins, and other contaminants are removed to yield high-quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization applications.

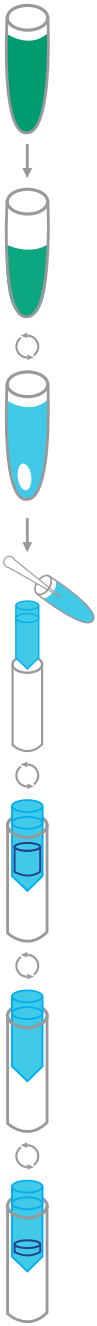
KIT CONTENTS

Product Number	26-022	26-023	26-023B
Purifications	5	50	50
Omni DNA Mini Columns	5	50	50
2 mL Collection Tubes	10	100	100
BB Buffer	5 mL	20 mL	20 mL
DW Buffer	1.5 mL	20 mL	20 mL
EB Buffer	2 mL	30 mL	30 mL
CTB Buffer	5 mL	40 mL	40 mL
Antifoam	1 mL		1 mL
2 mL Bead Kit 2.8 mm ceramic	5		50
User Manual	✓	✓	✓

STORAGE AND STABILITY

All of the Omni Plant DNA Purification Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in CTB Buffer and BB Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

ILLUSTRATED PROTOCOLS



Lyse and homogenize sample

Remove polysaccherides and polyphenols

Adjust the binding conditions

Transfer lysate into a DNA mini column to bind DNA

Wash column x 2

Dry the column

Elute DNA

BEFORE STARTING

This method is suitable for the isolation of DNA from up to 100 mg of fresh/frozen plant tissue and up to 30 mg of dried plant tissue.
Yields vary depending on source.

Materials and equipment to be supplied by user:

- Tabletop microcentrifuge capable of 10,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Waterbath or incubator capable of 65°C
- Bead Mill Homogenizer or vortexer
- β-mercaptoethanol (βME)
- Chloroform and isoamyl alcohol (24:1)
- Optional: RNase stock solution (20 mg/mL)
- 100% Ethanol

PREPARING REAGENTS

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

Kit	100% Ethanol to be added
26-022	6 mL
26-023 and 26-023B	80 mL

2. Prepare or obtain a mixture of chloroform: isoamyl alcohol (24:1)
3. β-mercaptoethanol (βME) must be added to CTB Buffer before use (20 µL βME/1 mL CTB).

PLANT DNA PURIFICATION KIT DIRECTIONS

26-023 - Tissue Digestion

1. Mince up to 100 mg fresh/frozen plant tissue (up to 30 mg dried tissue)
2. Transfer to a clean 1.5 mL microcentrifuge tube (not provided)
3. Add 500 µL CTB Buffer. Vortex vigorously to mix. Make sure to disperse all clumps.

NOTE: Ensure βME is added to CTB Buffer (20 µL βME/1 mL CTB).

Optional: Add 5 µL RNase. Let sit at room temperature for 5 minutes. Proceed to step 4.

4. Incubate at 65°C for 30 minutes. Invert the samples twice during incubation.

26-023B - Tissue Homogenization

1. Weigh up to 100 mg of fresh/frozen plant tissue (up to 30 mg dried tissue) and transfer to the 2 mL tube containing 2.8mm ceramic beads.
2. Add 500 µL of CTB buffer and 10 µL of Antifoam.

NOTE: Ensure βME is added to CTB Buffer (20 µL βME/1 mL CTB).

3. Dissociate sample on a bead mill at 3.5 – 4 m/s for 20 seconds or with continuous vortexing for 10 minutes.

Optional: After homogenization, add 5 µL RNase A. Let sit at room temperature for 5 minutes. Proceed to step 4.

4. Incubate at 65°C for 15 minutes. Invert the samples twice during incubation.

5. Add 700 µL chloroform/isoamyl alcohol (24:1). Vortex vigorously to mix.
6. Centrifuge at ≥10,000 x g for 5 minutes,
7. Transfer 300 µL aqueous phase (top) to a new microcentrifuge tube, making sure not to disturb the pellet or transfer any debris.
8. Add 150 µL BB Buffer and 300 µL 100% ethanol. Vortex to obtain a homogeneous mixture.

NOTE: A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

9. Insert an Omni DNA Mini Column into a 2 mL Collection Tube.
10. Transfer the entire sample (including any precipitate that may have formed) to the Omni DNA Mini Column.
11. Centrifuge at 10,000 x g for 1 minute.
12. Discard the filtrate and the Collection Tube.
13. Transfer the Omni DNA Mini Column to a new 2 mL Collection Tube.
14. Add 700 µL DW Buffer.

NOTE: DW Buffer must be diluted with 100% ethanol prior to use.

15. Centrifuge at 10,000 x g for 1 minute.
16. Discard the filtrate and reuse the Collection Tube.
17. Repeat Steps 14-16 for a second DW Buffer wash step.
18. Centrifuge the empty Omni DNA Mini Column at maximum speed for 2 minutes to dry the membrane.

NOTE: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.

19. Transfer the Omni DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).
20. Add 50 µL EB Buffer (or sterile deionized water) heated to 65°C.
21. Centrifuge at 10,000 x g for 1 minute.
22. Repeat Steps 20-21 for a second elution step.
23. Store DNA at -20°C.

TROUBLE SHOOTING GUIDE

Please use this guide to troubleshoot any problems that may arise.
For further assistance, please contact the technical support staff, toll free, at **1-800-776-4431.**

Problem	Cause	Suggestion
Clogged Column	Carry-over of debris.	Following extraction with chloro:isoamyl alcohol, make sure no particulate material is transferred.
	Sample too viscous.	Do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers CTB and BB and use two or more columns per sample.
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding CTB Buffer or use a Bead Ruptor if possible.
	Poor lysis of sample	Decrease amount of starting material or increase amount of CTB Buffer, chloro:isoamyl alcohol, and BB Buffer.
	DNA remains bound to column	Increase elution total volume to 200 µL and incubate at 65°C for 5 min before centrifugation.
	DNA washed off	Dilute DW Buffer by adding appropriate volume of absolute ethanol prior to use.
Problems in downstream applications	Salt carry-over	DW Buffer must be at room temperature.
	Ethanol carry-over	Following the second wash spin, ensure that the column is dried by centrifuging 2 min at maximum speed.

NOTES

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