Mag-Bind® Plant DNA Plus Kit

M1128-00	1 x 96 preps
M1128-01	4 x 96 preps

August 2013

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Manual Revision: August 2013

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The Mag-Bind® Plant DNA Plus 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 Omega Bio-tek's E.Z.N.A.® buffer chemistry with the convenience of Mag-Bind® 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, 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.

New in this Edition:

This kit has been improved to increase overall DNA extraction performance. This newly developed buffer system increases yield while decreasing contaminates and inhibitors.

Kit Contents

Product Number	M1128-00	M1128-01
Preparations	1 x 96	4 x 96
Mag-Bind® Particles CNR	1.7 mL	7 mL
CSPL Buffer	60 mL	240 mL
CSPW1 Buffer	39 mL	143 mL
CSPW2 Buffer	12 mL	44 mL
SPM Wash Buffer	36 mL	144 mL
Elution Buffer	15 mL	60 mL
RNase A	550 µL	2.2 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Plant DNA Plus Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Store Mag-Bind® Particles CNR and RNase A at 2-8˚C. All other materials should be stored at room temperature.

Preparing Reagents

1. Dilute CSPW1 Buffer with 100% ethanol as follows and store at room temperature.

CSPW1 Buffer	100% Ethanol to be Added
M1128-00	21 mL
M1128-01	77 mL

2. Dilute CSPW2 Buffer with isopropanol as follows and store at room temperature.

CSPW2 Buffer	Isopropanol to be Added
M1128-00	48 mL
M1128-01	176 mL

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

SPM Wash Buffer	100% Ethanol to be Added
M1128-00	84 mL
M1128-01	336 mL

Mag-Bind® Plant DNA Plus Kit - Fast Protocol for 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.

Materials and Equipment 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

Before Starting

- Prepare CSPW1 Buffer, CSW2 Buffer, and SPM Wash Buffer according to the instructions in the Preparing Reagents section on Page 4
- Set an incubator to 56°C
- Heat Elution Buffer to 65°C

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 CSPL 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.

   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. Let sit at room temperature for 10 minutes.

8. Add 400 µL isopropanol and 15 µL Mag-Bind® Particles CNR. Vortex to mix thoroughly.

9. Let sit at room temperature for 5 minutes.

10. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

11. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

12. Remove the plate from the magnetic separation device.

13. Add 500 µL CSPW1 Buffer. Vortex briefly or pipet up and down to resuspend the Mag-Bind® Particles CNR.

    Note: CSPW1 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 Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

16. Remove the plate from the magnetic separation device.

17. Add 500 µL CSPW2 Buffer. Vortex briefly or pipet up and down to resuspend the Mag-Bind® Particles CNR.

    Note: CSPW2 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 Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

19. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

20. Remove the plate from the magnetic separation device.

21. Add 500 µL SPM Wash Buffer. Vortex briefly or pipet up and down to resuspend the Mag-Bind® Particles CNR.

    Note: SPM Wash Buffer 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 Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

23. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

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

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

26. Remove the plate from the magnetic separation device.

27. Add 100 µL Elution Buffer heated to 65°C. Vortex briefly or pipet up and down to resuspend the Mag-Bind® Particles CNR.

28. Incubate at 65°C for 10 minutes.

29. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

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

31. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896.**

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA yields	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 SPM Wash Buffer by adding appropriate volume of ethanol prior to use (Page 4).
Problem	Cause	Solution
Problems in downstream applications	Salt carryover	SPM Wash Buffer must be at room temperature.
	Ethanol carryover	Dry the Mag-Bind® Particles CNR completely before adding elution buffer.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Elution Buffer (100 mL)	PDR048
SPM Wash Buffer (40 mL)	PS014
RNase A (5 mL)	AC118
96-well Microplate (500 µL) (5/pk)	EZ9604-01

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