Contents

Introduction

The E.Z.N.A.™ Mag-Bind™ Plant DNA Maxiprep Kit allows rapid and reliable isolation of high-quality genomic DNA from a wide variety of plant species and tissues. Up to 300 µg of high quality genomic DNA can be isolated from 1 gram of wet tissue (or 200 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the Mag-Bind™ particles with OBI's time proved efficiency of plant lysis buffer system to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. This kit is designed for preparation of high quality genomic, chloroplast, mitochondrial DNA. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

If using the E.Z.N.A.™ Mag-Bind™ Plant Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. Dry or fresh plant tissue is disrupted and then lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated. The lysate are transferred to a new tube and binding conditions are then adjusted so the genomic DNA will selected to bind with Mag-Bind™ particles. Two rapid wash steps remove trace contaminants such as residual polysaccharides and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

Most components of the E.Z.N.A.™ Mag-Bind™ Plant DNA Maxiprep Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. Silica-Magnetic particles solution should be stored at 4 °C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer SP1. It is possible to dissolve such deposits by warming the solution at 37°C, though we have found that they do not interfere with overall performance.

Kit Contents

Product Number	M2588-01	M2588-02
Purification Times	10 Preps	50 Preps
Homogenizer Maxi Column	10	50
50 ml Collection Tube	10	50
Magentic Particles C	2 x 1.1 ml	11 ml
Buffer SP1	70 ml	2 x 170 ml
Buffer SP2	25 ml	120 ml
Rnase A	1.1 ml	5.5 ml
MGB Binding Buffer	2 x 20 ml	4 x 50 ml
SPW Wash Buffer	60 ml	3 x 60 ml
Instruction Booklet	1	1

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.™ Mag-Bind™ Plant Maxi Kit procedure.

- Dilute MGB Binding Buffer with ethanol as follows and **store at room temperature**.

M2588-01	Add 80 ml absolute (96%-100%) ethanol to each bottle.
M2588-02	Add 200ml absolute (96%-100%) ethanol to each bottle.

- Dilute SPW Wash Buffer with absolute ethanol as follows and **store at room temperature**.

M2588-01	Add 140 ml absolute (96%-100%) ethanol.
M2588-02	Add 140 ml absolute (96%-100%) ethanol to each bottle.

E.Z.N.A®. Mag-bind® Plant DNA Maxi Magnetic Protocol

Materials to be provided by user:

- Centrifuge capable of 4000-5,000 x g with swinging-bucket rotor
- Nuclease-free 15 ml centrifuge tube.
- Water bath preset at 65°C
- Equilibrate sterile dH_2O water or 10 mM Tris pH 9.0 at 65°C.
- Absolute (96%-100%) ethanol
- Mortar and pestle (for manual tissue disruption)
- Tissue Lyser (for mechanic tissue disruption)

Grind Tissue

Manual disruption:
To prepare samples, collect tissue in a 30ml mortar and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using a clean pestles. Transfer the tissue powder and liquid nitrogen into a 15 ml centrifuge tube and allow the liquid nitrogen to evaporate. Immediately proceed the DNA isolation protocol.

Mechanic tissue disruption:
Place sample into a stainless steel grinding jar with appropriate steel beads. Frozen the stainless steel grinding jar in liquid nitrogen for 1 minutes and immediately attached the grinding jar into the clamps on the Tissuelyser. Grind tissue at 30Hz for 1-2 minutes.

1. **Collect 0.5-1g ground plant tissue in a 15 ml centrifuge tube which is capable of 5,000 x g and immediately add 6 ml Buffer SP1 and Vortex to mix.** Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue. Incubate at 65°C for 10 minutes. Mix sample few times during incubation by inverting tube.

2. **Add 2 ml Buffer SP2 and vortex to mix**. Centrifuge at 4000-5000 x g in a swinging-bucket centrifuge for 15 min to pellet cell debris.

3. **Carefully aspirate supernatant to a Homogenizer Maxi Column (supplied) placed in a 50 ml centrifuge tube**. Centrifuge at 4000-5000 x g for 10 minutes at room temperature in a swinging-bucket centrifuge.

4. Transfer the flow-through without disturbing the pellet in the collection tube to a new 50 ml centrifuge tube (not supplied).

5. Add 0.1 ml RNase A and mix well. Incubate at room temperature for 30-60 minutes.

6. **Add 1 volume of MGB Binding Buffer and 200µl Magnetic particle solution directly into the cleared lysate and mix immediately by vortexing.**
Note: 1). Dilute MGB Binding Buffer with absolute ethanol before use. 2). Silica-Magnetic Particle has to be fully suspended by shaking or vortexing before use.

7. **Incubate the sample at room temperature for 5 minutes.**

8. Place the tube on a magnetic separation device suitable for 50 ml tube to magnetize the Magnetic particles. Remove and discard the cleared supernatant.

9. Remove the tube containing the Magnetic particles from the magnetic separation device. **Add 5 ml of SPW Wash Buffer diluted with ethanol into the tube.**

10. **Resuspend Magnetic particles pellet by vortexing.** Incubate 3 minutes at room temperature. Repeating the mix by vortexing for 1 minutes.

11. Place the plate onto a magnetic separation device to magnetize the Mag-Bind™ particles. Remove and discard the cleared supernatant.

12. Remove the tube containing the Magnetic particles from the magnetic separation device. **Add 5 ml SPW Wash Buffer diluted with ethanol into the tube.**

13. **Resuspend Magnetic particles pellet by vortexing.** Incubate 3 minutes at room temperature. Repeating the mix by vortexing for 1 minutes.

14. Place the plate onto a magnetic separation device to magnetize the Silica-Magnetic particles. Remove and discard the cleared supernatant.

15. Leave the tube to air dry on the magnetic separation device for 5-10 minutes. Remove any residue liquid from tube by pipetting.

16. Remove the tube from magnetic separation device. **Add 0.2-1 ml Elution Buffer or water to elute DNA from magnetic particles.**

17. **Resuspend Magnetic particles by Vortexing.** Incubate 15 minutes at 65°C.

Repeating the mix by vortexing.

18. Place the tube onto a magnetic separation device to magnetize the Silica-Magnetic particles.

19. **Transfer the cleared supernatant containing purified DNA to a new 1.5 ml tube.**

E.Z.N.A®. Mag-bind® Plant DNA Maxi Spin Protocol

E.Z.N.A®. Mag-bind® Plant DNA Maxi Kit can be proceeded by spinning if without magnetic separation devices available. The Purification of genomic DNA isolated by spin protocol may be lower than the Magnetic protocol because some unsoluble materials can be precipitate with DNA.

1. **Following Step 1-7 of Magnetic Protocol on page 4-5.**

2. **Centrifuge at 4,000 x g for 10 min at room temperature.** Remove and discard the cleared supernatant.

3. **Add 5 ml of SPW Wash Buffer diluted with ethanol into the tube.** Resuspend Magnetic particles pellet by vortexing. Incubate 3 min at room temperature.

4. **Centrifuge at 4000 x g for 10 min at room temperature.** Remove and discard the cleared supernatant.

5. Repeat step 3-4 one time.

6. Air dry for 10-15 min. Remove any residue liquid from tube by pipetting.

7. **Add 0.2-1 ml Elution Buffer or water to elute DNA from magnetic particles.**

8. **Resuspend Magnetic particles by vortexing.** Incubate for 15 minutes at 65°C. Repeating the mix by vortexing.

9. Centrifuge at 4000 x g for 15 min. Transfer the supernatant into a new tube.

Troubleshooting

Problem	Cause	Suggestions
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer SP1.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers SP1 and SP2.
	Loss the Silica-Magnetic particle during operation	Carefully avoid remove the SMagnetic particles during aspiration
	DNA remains bound to Silica-Magnetic Particles	Increase elution volume and incubate on column at 65°C for 5 min elution
	DNA washed off.	Dilute MGC Binding Buffer and MGW Wash Buffer by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications	Salt carry-over.	Wash Buffer must be at room temperature.
	Ethanol carry-over	Dry the Magnetic particle before elution.