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Revised August 2006

Introduction

The E.Z.N.A.™ Mag-Bind® Plant DNA Maxi 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) in less than 1 hour. The system combines the reversible nucleic acid-binding properties of Mag-Bind® particles with the time-proven efficiency of OBI's 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, and 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 DNA Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Dry or fresh plant tissue is disrupted and then lysed in a specially formulated buffer. Proteins, polysaccharides, and cellular debris are subsequently precipitated. The lysate is transferred to a new tube and binding conditions are adjusted so that genomic DNA will selectively bind to the Mag-Bind®particles. Two rapid wash steps remove trace contaminants such as residual polysaccharides; then pure DNA is eluted in Elution 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 Maxi Kit are stable for at least 24 months from date of purchase when stored at 22ºC-25ºC. Mag-Bind® Particles Solution B should be stored at 4° C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer SP1. Dissolve such deposits by warming the solution at 37ºC and gently shaking.

Kit Contents

Product Number	M2588-00	M2588-01	M2588-02
Purification	2	10	50
Mag-Bind® Particles Solution B	210 µL	1.1 mL	5.5 mL
Maxi-Homogenizer Column	2	10	50
50mL Centrifuge Tube	2	10	50
Buffer SP1	20 mL	100 ml	500 mL
Buffer SP2	8 mL	35 mL	180 mL
MGB Binding Buffer	5 mL	30 mL	140 mL
SPM Wash Buffer	15 mL	75 mL	3 x 150 mL
RNase A	50 µL	250 µL	1.2 mL
Elution Buffer	5 mL	20 mL	50 mL
Instruction Booklet	1	1	1

Before Starting

Please read this booklet thoroughly to become familiar with the E.Z.N.A.™ Mag-Bind® Plant DNA Maxi Kit procedures.

- **Elution Buffer (or sterile water) at 65°C.**

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

M2588-00	Add 20 mL absolute ethanol (96%-100%)
M2588-01	Add 120 mL absolute ethanol (96%-100%)
M2588-02	Add 560 mL absolute ethanol (96%-100%)

- Dilute **SPM Wash Buffer** with **absolute ethanol** (96%-100%) as follows and **store at room temperature**.

M2588-01	Add 35 mL absolute ethanol (96%-100%)
M2588-01	Add 175 mL absolute ethanol (96%-100%)
M2588-02	Add 350 mL absolute ethanol (96%-100%)

Mag-Bind® Plant DNA Maxi Kit Protocol

Materials to be provided by user

- Centrifuge capable of 4,000-5,000 x g with swinging-bucket rotor
- Nuclease-free 15 mL centrifuge tubes
- Nuclease-free 50 mL centrifuge tubes
- Water bath preset at 65°C
- Absolute ethanol (96%-100%)
- Equipment for disrupting plant tissue (MM300 Mixer Mill or Geno/Grinder 2000)
- Mortar and pestle (for manual tissue disruption)
- Tissue Lyser (for mechanical tissue disruption)
- Magnetic stand for 15 mL tube

Tissue Disruption

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

Mechanical tissue disruption:
Place sample into a stainless steel grinding jar with appropriate steel beads. Freeze samples in the stainless steel grinding jar using liquid nitrogen for 1 minute. Immediately attach the grinding jar onto the clamps of the Tissuelyser. Grind tissue at 30 Hz for 1-2 minutes.

1. Collect 500 mg (dried) or (2.5 gram) ground plant tissue in a 15 mL centrifuge tube capable of withstanding 5,000 x g. Immediately add 9 mL Buffer SP1 and 20 µL RNase A. Incubate at 65°C for 10 minutes. Mix sample several times during incubation by inverting tube. *Be sure to disperse all clumps;*

DNA cannot be effectively extracted from clumped tissue.

Note: Longer incubation time in Buffer SP1 (15 to 20 min) will yield better results for some plant specimens. Increase time according to results.

2. Add 3 mL Buffer SP2 and vortex to mix throughly. Incubate the tube on ice for 10 minutes.

3. Centrifuge at 4,000-5,000 x g in a swinging-bucket centrifuge for 10 min to pellet cell debris.

4. Carefully aspirate supernatant to a Maxi Homogenizer Column (supplied) placed in a 50 mL centrifuge tube. Make sure not to disturb the pellet or transfer any debris.

5. Centrifuge at 4,000 x g for 5 minutes at room temperature in a swinging-bucket centrifuge.

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

7. Add 1 volume MGB Binding Buffer and 100 µL Mag-Bind® Particles Solution B directly into the cleared lysate and mix immediately by vortexing.

   Note: 1. Dilute MGB Binding Buffer with absolute ethanol before use. See Page 3 or bottle label for instructions. **2.** Mag-Bind® Particles Solution B will bead together in its container. It must be fully resuspended by shaking or vortexing before use. **(IMPORTANT)**

8. Incubate the sample at room temperature for 5 minutes.

9. Place the tube on a magnetic separation device suitable for 50 mL tubes to magnetize the Mag-Bind® particles. The solution will clear when the particles have migrated toward the magnetic source.

10. Remove and discard the cleared supernatant.

11. Remove the tube containing the Mag-Bind® particles from the magnetic separation device. Add 5 mL SPM Wash Buffer diluted with absolute ethanol into the tube.

12. Resuspend the Mag-Bind® particles pellet by vortexing. Incubate 3 minutes at room temperature. Repeat the mix by vortexing for 30-50 seconds.

13. Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles.

14. Remove and discard the cleared supernatant.

15. Remove the tube containing the Mag-Bind® particles from the magnetic

separation device. Add 5 mL SPM Wash Buffer diluted with absolute ethanol into the tube.

16. Resuspend the Mag-Bind® particles pellet by vortexing. Incubate 3 minutes at room temperature. Repeating the mix by vortexing for 30-50 seconds.

17. Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles.

18. Remove and discard the cleared supernatant.

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

    Note: It is critical that the Mag-Bind® particles are completely dried for elimination of trace ethanol that might otherwise interfere with downstream applications. Avoid over-drying the particles, however, which can make resuspension difficult in the following elution steps.

20. Remove the tube from magnetic separation device. Add 200-500 ul Elution Buffer to elute the DNA from the Mag-Bind® particles.

21. Resuspend the Mag-Bind® particles by vortexing. Incubate 5 minutes at room temperature. Repeating the mix by vortexing for 30-50 seconds.

22. Place the tube onto a magnetic separation device to magnetize the Mag-Bind® particles.

23. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL tube.

Mag-Bind® Plant DNA Maxi Kit Protocol (Centrifugation)

The following protocol is designed for isolating genomic DNA from up to 500 mg (dried sample) or 2.5 gram (frozen or fresh) plant sample using Mag-Bind particles with centrifugation.

1. Disrupt and homogenize the sample as described on page 4.

2. Collect 500 mg (dried) or (2.5 gram) ground plant tissue in a 15 mL centrifuge tube capable of withstanding 5,000 x g. Immediately add 9 mL Buffer SP1 and 50 µL RNase A. Incubate at 65°C for 10 minutes. Mix sample several times during incubation by inverting tube. *Be sure to disperse all clumps; DNA cannot be effectively extracted from clumped tissue*

   Note: Longer incubation time in Buffer SP1 (15 to 20 min) will yield better results for some plant specimens. Increase time according to results.

3. Add 3 mL Buffer SP2 and vortex to mix throughly. Incubate the tube on ice for

10 minutes.

4. Centrifuge at 4,000-5,000 x g in a swinging-bucket centrifuge for 10 min to pellet cell debris.

5. Carefully aspirate supernatant to a Maxi Homogenizer Column (supplied) placed in a 50 mL centrifuge tube. Make sure not to disturb the pellet or transfer any debris.

6. Centrifuge at 4,000 x g for 5 minutes at room temperature in a swinging-bucket centrifuge.

7. Add 1 volume MGB Binding Buffer and 100 µL Mag-Bind® Particles Solution B directly into the cleared lysate and mix immediately by vortexing.

   Note: 1. Dilute MGB Binding Buffer with absolute ethanol before use. See Page 3 or bottle label for instructions. **2.** Mag-Bind® Particles Solution B will bead together in its container. It must be fully resuspended by shaking or vortexing before use. **(IMPORTANT)**

8. Incubate the sample at room temperature for 5 minutes

9. Centrifuge at 5000 x g for 5 minutes to pellet the magnetic particles.

10. Discard the supernatant and add 10 mL SPM Buffer into the tube. Mix throughly by vortexing.

11. Centrifuge at 5000 x g for 5 minutes to pellet the magnetic particles.

12. Discard the supernatant and dry the pellet by invert the tube on a absorbent paper for 10-20 minutes. Remove any liquid on the wall of the tube. **It is critical to completely dry the pellet before the elution because the residual SPM buffer contains ethanol that will interfere many downstream applications.**

13. Add 200-500 ul Elution Buffer to elute the DNA from the Mag-Bind® particles. Resuspend the pellet by vortexing or pipetting.

14. Centrifuge at 5000 x g for 5 minutes to pellet the magnetic particles. Transfer the eluted DNA to a new 1.5 mL tube.

Yield and quality of DNA: determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$DNA\ concentration = Absorbance_{260} \times 50 \times (Dilution\ Factor)\ \mu g/mL$$

The ratio of ($absorbance_{260}$)/($absorbance_{280}$) is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid. Alternatively,

quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations.

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 of Mag-Bind® particles during operation	Carefully avoid remove the Mag-Bind® particles during aspiration
	DNA remains bound to Mag-Bind® Particles	Increase elution volume and incubate at 65°C for 5 min prior to removal from magnetic particles.
	DNA washed off	Dilute MGB Binding Buffer and SPM Wash Buffer by adding appropriate volumes of absolute ethanol prior to use (page 3).
Problems in downstream applications	Salt carry-over	SPM Wash Buffer must be at room temperature.
	Ethanol carry-over	Dry the Mag-Bind® particles before elution.