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Revised June 2009

Introduction

E-Z 96® Mag-Bind® Plant DNA Kits allow rapid and reliable isolation of high-quality genomic DNA from a wide variety of plant species and tissues. Up to ninety-six 100 mg samples of wet tissue (or 30 mg dry tissue) per microplate can be processed in less than 1 hour. The system combines Omega Bio-Tek's EaZy Nucleic Acid® 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 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 96® Mag-Bind® Plant DNA Kit for the first time, please read this booklet to become familiar with the procedures. 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 is transferred to a 96-well microplate, binding conditions are adjusted, and genomic DNA binds to the Mag-Bind® particles. One or two rapid wash steps remove trace contaminants such as residual polysaccharides. And finally 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

All components of the E-Z 96® Mag-Bind® Plant DNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. For long term use, store Mag-Bind® Particles Solution at 2°C-8°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.

Kit Contents

Product	M1127-01	M1127-02	M1127-03
Purification	2 x 96	8 x 96	20 x 96
Mag-Bind Particles V	2.2 mL	17 mL	44 mL
Buffer SP1	170 mL	700 mL	2 x 850 mL
Buffer SP2	45 mL	175 mL	875 mL
SPM Buffer	72 mL	144 mL	3 x 300 mL
Buffer MGB	25 mL	50 mL	5 x 250 mL
Elution Buffer	30 mL	125 mL	300 mL
Rnase A	800 µL	4 x 800 µL	8 mL
User Manual	1	1	1

NOTE: The E.Z.N.A.® Mag-Bind Plant DNA Isolation Kit is supplied with enough buffer for the standard protocol. However, due to increased volumes called for in some protocols, fewer preparations may be performed. Also, additional buffers can be purchased separately from Omega Bio-Tek.

Preparing Reagents

Important	SPM Buffer must be diluted with absolute ethanol as follows **M1127-00** Add 240 mL ethanol / bottle **M1127-01** Add 336ml ethanol/bottle **M1127-02** Add 700 mL ethanol / bottle
	Buffer MGB Must be diluted with absolute ethanol as follows **M1127-00** Add 100 mL ethanol / bottle **M1127-01** Add 200ml ethanol/bottle **M1127-02** Add 200 mL ethanol / bottle

Plant DNA Protocol Dry Specimens
KingFisher 96 Process

Pipetting Instruction for KingFisher 96 and Mag-Bind Plant DNA protocol

Plate Type	Plate	Content	Reagent Volume
A	1	Sample/ Lysate according to protocols below.	920 µl
A	2	SPM Buffer	600 µl
A	3	SPM Buffer	600 µl
B	4	Elution Buffer	100 µl
A	5	Tip Loading Plate	

* A= KingFisher 96 DW Plate, B=KingFisher 96 KF Plate

- Prepare lysate by following the use instruction based on sample type.
- Add 600 µl SPM Wash Buffer to **Plate 2.**
- Add 600 µl SPM Wash Buffer to **Plate 3**.
- Add 100 µl Elution Buffer to **Plate 4.**
- Add Kingfisher 96 Tip for Deep Well Magnets Sleeve to **Plate 5.**

Materials to be provided by user:

- Centrifuge capable of at least 3,000-5,000 x g
- Rotor adapter for 96-well microplate
- Incubator equilibrated to 65°C
- Absolute (96%-100%) ethanol
- Equipment for disrupting plant tissue (MM300 Mixer Mill or Geno/Grinder 2010 or Geno/Grinder 2000 and Tungsten carbide beads)
- 8- or 12-channel pipette
- Reagent reservoir for pipette
- Ice Bucket
- Kingfisher 96 or Kingfisher Flex 96 with Deep Well Magnet
- 4 Deep Well Kingfisher 96 Plates
- 1 Kingfisher 96 Plate
- 1 Tip Sleeve for Kingfisher 96 Deep Well Magnet
- Sealing Film

This is the most robust method for isolation of total cellular (mitochondrial, chloroplast, and genomic) DNA. Drying allows storage of field specimens for prolonged periods of time prior to processing. Samples can be dried overnight in a 45°C oven, powdered, and stored dry at room temperature.

1. To prepare dried samples, place 10-30 mg of dried tissue into a deep well plate or 1.2 mL microtube rack in the presence of Buffer SP1 and grinding bead. For 10-30 mg dry sample add 800µl Buffer SP1 and 4 µL of RNase A.

   Note: SP1 Buffer and RNase A can be combined in appropriate proportions to make a master mix before starting the procedure. RNase A activity is lost after longterm storage in Buffer SP1.

2. Process in the mixer mill machine by following manufacturer's instructions. Time and speed will need to be determined for each type of sample.

3. Incubate at 65°C for 10 min following sample distruption. Mix sample twice during incubation by inverting tube or vortexing plate very briefly.

4. Add 280 µL Buffer SP2 and vortex to mix for 10 seconds. Incubate the tube rack for 10 minutes at -20°C. This step helps to remove the proteins, polysacchrides and other inhibitors.

5. Centrifuge at 3,000-5,000 x g (5,000 x g is better, if available) for 10 min. Compact pellets will form at bottom of tubes but some particles may float. Be careful to avoid those particles during the transfer in next step.

6. Carefully transfer 450µl supernatant to a 96-well Kingfisher Deep Well Plate, making sure not to disturb the pellet or transfer any debris.

7. Add 20µl/well of Mag-Bind® Particles Solution V; follow by addition of 450µl/well of MGB Binding Buffer.
   Tip: MGB Binding Buffer and Mag-Bind® Particles Solution can be combined in appropriate proportions to make a master mix before starting the procedure. Add 470 µL per well of Mag-Bind® Particle Solution B/MGB Binding Buffer master mix to each well of the microplate.

8. Press Start on the Mag Bind Plant DNA Protocol and load plates according to prompts from Kingfisher Unit

9. Seal Plate with Sealing Film (not provided) and store purified DNA at - 20°C

Plant DNA Protocol Fresh/Frozen Specimens

Pipetting Instruction for KingFisher 96 and Mag-Bind Plant DNA protocol

Plate Type	Plate	Content	Reagent Volume
A	**1**	**Sample/ Lysate according to protocols below.**	**820 µl**
A	**2**	**SPM Buffer**	**600 µl**
A	**3**	**SPM Buffer**	**600 µl**
B	**4**	**Elution Buffer**	**100 µl**
A	**5**	**Tip Loading Plate**	

1. Prepare lysate by following the use instruction based on sample type.
2. Add 600 µl SPM Wash Buffer to **Plate 2.**
3. Add 600 µl SPM Wash Buffer to **Plate 3**.
4. Add 100 µl Elution Buffer to **Plate 4.**
5. Add Kingfisher 96 Tip for Deep Well Magnets Sleeve to **Plate 5**

Materials to be provided by user:

- Micro centrifuge capable of at least 10,000 x g
- Centrifuge capable of at least 3,000-5,000 x g
- Rotor adapter for 96-well microplate
- Incubator equilibrated to 65°C
- Absolute (96%-100%) ethanol
- Equipment for disrupting plant tissue (MM300 Mixer Mill or Geno/Grinder 2000 and Tungsten carbide beads) or Liquid Nitrogen
- 8- or 12-channel pipette
- Reagent reservoir for pipette
- Ice Bucket
- 1.5 or 2.0 mL microcentrifuge tubes, sealed deep-well plate or capped microtube rack for sample disruption
- Kingfisher 96 or Kingfisher Flex 96 with Deep Well Magnet
- 4 Deep Well Kingfisher 96 Plates
- 1 Kingfisher 96 Plate
- 1 Tip Sleeve for Kingfisher 96 Deep Well Magnet
- Sealing Film

Note: Use extreme caution when handling liquid nitrogen.

This protocol is suitable for most fresh or frozen tissue samples allowing more efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to ≤100 mg. Best results are obtained with young leaves or needles.

1. Collect plant tissue in a microfuge tube or deepwell plate (not supplied). Grind the samples as discussed below.

   A. To prepare samples collect tissue in a 1.5 mL or 2 mL microfuge tube and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable pellet pestles, which are available from Omega Bio-Tek (Cat# SS1015-39). Add 300µl SP1 Buffer and 1µL of RNase A immediately. Mix by vortexing. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70oC for later use. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and carefully wiping the surfaces clean between samples.

   B. To prepare samples in 96-well plate format, place fresh leaf disks in a sealed 96-well deep-well plate or capped microtube rack in the presence of 600 µL of Buffer SP1 and 4 µL RNase A with one or two grinding beads. Process in the MM300 Mixture Mill or Geno/Grinder Mixture Mill following the manufacturer's instructions.

   Note: Processing time and speed of the Mixture Mill will need to be determined for each sample type. Use the minimium speed that produces an even suspension to prevent excess shearing of the DNA.

   TIP:: SP1 Buffer and RNase A can be combined in appropriate proportions to make a master mix before starting the procedure. RNase A activity is lost after longterm storage in Buffer SP1.

2. Incubate at 65°C for 10 min. Mix sample twice during incubation by inverting tube or vortexing plate very briefly.

3. Add 220 µL Buffer SP2 and vortex to mix. Incubate on ice for 5 minutes.

4. Centrifuge at 3,000-5,000 x g (5,000 x g is better, if available) for 10 min. Compact pellets will form at bottom of tubes but some particles may float. Be careful to avoid those particles during the transfer in next step.

5. Carefully aspirate 400 µL cleared lysate to a 500 µL Kingfisher 96-well Deep Well Plate, making sure not to disturb the pellet or transfer any debris.

6. Add 20 µL/well of Mag-Bind® Particle Solution B; follow by addition of 400µL/well of MGB Binding Buffer.

   NOTE: The Mag-Bind® Particles will settle and bead together at the bottom of their container. Please check container before use. If beading has occurred, gently shake or vortex container until particles have been redispersed in solution. (IMPORTANT)

   Tip: MGB Binding Buffer and Mag-Bind® Particles Solution can be combined in appropriate proportions to make a master mix before starting the procedure. Add 420 µL per well of Mag-Bind® Particle Solution B/MGB Binding Buffer master mix to each well of the microplate.

7. Press Start on the Mag Bind Plant DNA Protocol and load plates according to Prompts

8. Seal Plate with Sealing Film (not provided) and store purified DNA at -20°C

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. DNA concentration is calculated as:

[DNA] = (Absorbance$_{260}$) x (0.05 µg/ µL) x (Dilution factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity.

Expected yields vary with both amount, and type of tissue used. 30 mg of fresh tissue will yield 10-40 µg DNA

Troubleshooting Guide

Problem	Cause	Suggestions
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples make sure grind tissue completely.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers SP1 and SP2.
	DNA remains bound to magnetic beads	Increase elution volume to 100 µL and incubate at 65°C for 5 min before separating eluate.
	DNA washed off.	Dilute SPM Wash Buffer by adding appropriate volume of absolute ethanol prior to use (Page 3).
Problems in downstream applications	Salt carry-over.	SPM Bufffer must be at room temperature.
	Ethanol carry-over	Dry the magnetic beads pellet completely before adding elution buffer.