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Introduction

The E.Z.N.A.® Mag-Bind Plant DNA Kit allows rapid and reliable isolation of high-quality genomic DNA from a wide variety of plant species and tissues. Up to 10 µg of high quality genomic DNA can be isolated from 50 mg of wet tissue (or 10 mg dry tissue) and can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of MagSi 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. The lysate is transferred to a new plate and binding conditions are adjusted so that genomic DNA will selectively bind to the MagSi. Four rapid wash steps remove trace contaminants such as residual polysaccharides, and pure DNA is eluted in Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Kit Contents

Product Number	M1027-01	M1027-02
Purificatios	2 x 96 Preps	8 x 96 Preps
MagSi Particles	5 ml	18 ml
Buffer SLX Minus	200 ml	4 x 200 ml
Buffer DS	10 ml	40 ml
Buffer PHB	250 ml	4 x 250 ml
SPM Wash Buffer	75 ml	4 x 75 ml
RNase A	1.0 ml	4 x 1.0 ml
Elution Buffer	25 ml	4 x 25 ml
Instruction Booklet	1	1

Storage and Stability

Most components of the E.Z.N.A.® Mag-Bind Plant DNA Kit, except RNase A are stable for at least 24 months from date of purchase when stored at 22-25ºC. MagSi Particles Solution should be stored at 4° C for long-term use. Store RNase A at -20° C.

Before Starting

- Please read this booklet thoroughly to become familiar with the E.Z.N.A.® MagSi Plant DNA Kit procedures.
- Dilute SPM Wash Buffer with absolute ethanol as follows and **store at room temperature**.

M1027-01	Add 175 ml absolute (96%-100%) ethanol
M1027-02	Add 175 ml absolute (96%-100%) ethanol / bottle

Materials to be provided by user

- Centrifuge capable of 4,000 x g.
- 96 well Plate
- Water bath preset at 65-70°C
- Absolute (96%-100%) ethanol
- Equipment for disrupting plant tissue (MM300 Mixer Mill or Geno/Grinder 2000)

Mag-Binds Plant DNA Kit Magnetic 96 Protocol

Tissue Disruption

Manual disruption:

To prepare samples, collect plant sample 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 sample powder and liquid nitrogen into 1.2 ml 96 plate 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 30Hz for 1-2 minutes.

1. Collect ground plant tissue (start with 50mg for fresh sample and 10mg for dried samples) in a 1.2 ml 96 well plate. **Immediately add 600µl Buffer SLX Minus and 4µl RNase A.** Shaking vigorously to disperse all clumps.

   Note: Buffer SLX Minus 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 SLX Minus.

2. **Incubate at 70°C for 10 minutes.** Mix sample once during incubation by shaking.

3. **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.

4. **Carefully transfer 400 µl supernatant to 2ml Deep Well Plate, making sure not to disturb the pellet or transfer any debris.**

5. **Add 20 µl MagSi Particles and 280 µl absolute thanol to each well.** Shaking to mix for 2 min.

   Note: Absolute ethanol and MagSi Particles can be premix. MagSi Particles

will bead together in its container after several minutes It must be fully suspended by shaking or vortexing before use. **(IMPORTANT)**

6. Place the plate on a magnetic separation device to magnetize the MagSi particles for 2-5 minutes . Remove and discard the cleared supernatant.

7. Remove the plate containing the MagSi particles from the magnetic separation device. **Add 500µl Buffer PHB to each well.**

8. Resuspend MagSi particles pellet by shaking for 2min. Place the tube on a magnetic separation device to magnetize the MagSi particles for 2-5 minutes . Remove and discard the cleared supernatant.

9. Remove the plate containing the MagSi particles from the magnetic separation device. **Add 500µl Buffer PHB to each well.**

10. Resuspend MagSi particles pellet by shaking for 2min. Place the tube on a magnetic separation device to magnetize the MagSi particles . Remove and discard the cleared supernatant.

11. Remove the plate containing the MagSi particles from the magnetic separation device. **Add 500µl SPM Wash Buffer to each well.**

12. Resuspend MagSi particles pellet by shaking for 1 min. Place the tube on a magnetic separation device to magnetize the MagSi particles . Remove and discard the cleared supernatant.

13. Remove the plate containing the MagSi particles from the magnetic separation device. **Add 500µl SPM Wash Buffer to each well.**

14. Resuspend MagSi particles pellet by shaking for 1 min. Place the tube on a magnetic separation device to magnetize the MagSi particles . Remove and discard the cleared supernatant.

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

16. Remove the tube from magnetic separation device. Add 50-100ul Elution Buffer or water to elute DNA from the magnetic particles.

17. Resuspend MagSi particles by shaking for 1 min. Incubate 5 min at room

temperature (best at 55-65°C). Shaking for 3 minutes.

18. Place the tube onto a magnetic separation device to magnetize the MagSi particles. Transfer the cleared supernatant containing purified DNA to a new plate.

Mag-Binds Plant DNA Kit Magnetic Fast Protocol

1. Collect plant tissue (start with 50mg for fresh sample and 10mg for dried samples) in a 1.2 ml 96 well plate with appropriate steel beads. **Add 600µl Buffer SLX Minus and 4µl RNase A.**

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 70°C for 10 minutes.** Mix sample once during incubation by shaking.

4. **Centrifuge at 3,000-5,000 x g (5,000 x g is better, if available) for 15 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 transfer 400 µl supernatant to 2ml Deep Well Plate, making sure not to disturb the pellet or transfer any debris.**

6. **Add 20 µl MagSi Particles and 280 µl absolute thanol to each well.** Shaking to mix for 2 min.
   Note: Absolute ethanol and MagSi Particles can be premix. MagSi Particles will bead together in its container after several minutes It must be fully suspended by shaking or vortexing before use.

7. Place the plate on a magnetic separation device to magnetize the MagSi particles for 2-5 minutes . Remove and discard the cleared supernatant.

8. Remove the plate containing the MagSi particles from the magnetic separation device. **Add 500µl SPM Wash Buffer to each well.**

9. Resuspend Magsi particles pellet by shaking for 1 min. Place the tube on a magnetic separation device to magnetize the MagSi particles . Remove and discard the cleared supernatant.

10. Remove the plate containing the MagSi particles from the magnetic separation device. **Add 500µl SPM Wash Buffer to each well.**

11. Resuspend MagSi particles pellet by shaking for 1 min. Place the tube on a magnetic separation device to magnetize the MagSi particles . Remove and discard the cleared supernatant.

12. Leave the plate to air dry on the magnetic separation device for 10min. Remove any residue liquid from tube by pipetting.

13. Remove the tube from magnetic separation device. **Add 50-100ul Elution Buffer or water to elute DNA from the magnetic particles.**

14. **Resuspend MagSi particles by shaking for 1 min. Incubate 5 min at room temperature (best at 55-65°C). Shaking for 3 minutes.**

15. Place the tube onto a magnetic separation device to magnetize the MagSi particles. Transfer the cleared supernatant containing purified DNA to a new plate.

Mag-Binds Plant DNA Protocol For RICE Powder or other foods

1. Collect 100mg rice or rice powder in a 2 ml 96 well plate with appropriate steel beads. **Add 1ml Buffer SLX Minus and 4µl RNase A.**

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. **Add 50 µl Buffer DS (and optional: 20 µl Proteinase K) to the lysate.** Mix well by shaking. Proteinase K (20mg/ml) can increase the sensitive.

4. **Incubate at 65°C for 30-60 minutes.** Mix sample several times during incubation by shaking.

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 200 µl supernatant to 0.5 ml Plate,** making sure not to disturb the pellet or transfer any debris.

7. **Add 20 µl Magsi Particles and 150 µl absolute thanol to each well.** Mix well by pipetting up and down 50 times. Incubate at room temperature for 3 min.
   Note: Absolute ethanol and MagSi Particles can be premix. MagSi Particles will bead together in its container after several minutes It must be fully suspended by shaking or vortexing before use.

8. Place the plate on a magnetic separation device to magnetize the MagSi particles . Remove and discard the cleared supernatant.

9. Remove the plate containing the MagSi particles from the magnetic separation device. **Add 400µl SPM Wash Buffer to each well.**

10. Resuspend MagSi particles pellet by by pipetting up and down 30 times. Place the tube on a magnetic separation device to magnetize the MagSi particles . Remove and discard the cleared supernatant.

11. Remove the plate containing the MagSi particles from the magnetic separation device. **Add 400µl SPM Wash Buffer to each well.**

12. Resuspend Magsi particles pellet by by pipetting up and down 30 times.. Place the tube on a magnetic separation device to magnetize the Mag-Si particles . Remove and discard the cleared supernatant.

13. Leave the plate to air dry on the magnetic separation device for 10min. Remove any residue liquid from tube by pipetting.

14. **Remove the tube from magnetic separation device. Add 30-50ul Elution Buffer or water to elute DNA from the magnetic particles.**

15. Resuspend MagSi particles by by pipetting up and down 30 times. Incubate 5 min at room temperature (best at 55-65°C). Mix well by pipetting up and down 30 times

16. Place the tube onto a magnetic separation device to magnetize the MagSi particles. Transfer the cleared supernatant containing purified DNA to a new plate.

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 SLX Minus Buffer.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers SLX Minus.
	Loss the MagSi particle during operation	Carefully avoid removing the MagSi particles during aspiration
	DNA remains bound to MagSi Particles	Increase elution volume and incubate at 65ºC for 5 min elution
	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 Wash Buffer must be at room temperature.
	Ethanol carry-over	Dry the MagSi particle before elution.