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## Introduction

E.Z.N.A.® MagBeads® 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 gram of wet tissue (or 10 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of MagBeads® particles with the time-proven efficiency of OBI's SP 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.® MagBeads® 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 MagBead®. Two 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.

## Storage and Stability

Most components of the E.Z.N.A.® MagBead® Plant DNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. MagBead® Particles Solution X 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 37°C and gently shaking.

## Kit Contents

Product Number	M2327-00	M2327-01	M2327-02
Purifications	5	50	200
MagBead <sup>®</sup> Solution S	1ml	9 ml	36 ml
Buffer SP1	5 ml	25 ml	100 ml
Buffer SP2	1.0 ml	10 ml	40 ml
MGW Wash Buffer	1.5 ml	15 ml	60 ml
RNase A	12µl	120µl	440µl
Elution Buffer	600µl	10 ml	40 ml
Instruction Booklet	1	1	1

## Before Starting

- Please read this booklet thoroughly to become familiar with the E.Z.N.A.<sup>®</sup> MagBeads<sup>®</sup> Plant DNA Kit procedures.
- Equilibrate Buffer SP1 and Elution Buffer ( or sterile dH<sub>2</sub>O water or 10 mM Tris pH 9.0) at 65°C.
- Dilute MagBead Solution S with absolute ethanol as follows and **store at room temperature**.

<b>M2327-00</b>	Add 4 ml absolute (96%-100%) ethanol.
<b>M2327-01</b>	Add 36ml absolute (96%-100%) ethanol to each bottle.
<b>M2327-02</b>	Add 144 ml absolute (96%-100%) ethanol to each bottle.

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

<b>M2327-00</b>	Add 3.5 ml absolute (96%-100%) ethanol.
<b>M2327-01</b>	Add 35 ml absolute (96%-100%) ethanol
<b>M2327-02</b>	Add 140 ml absolute (96%-100%) ethanol

## MagBeads<sup>®</sup> Plant DNA Maxiprep Kit Protocol

Materials to be provided by user:

- Centrifuge capable of 12,000 x g.
- Nuclease-free 15 ml centrifuge tube.
- Water bath preset at 65°C
- Absolute (96%-100%) ethanol
- 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)

## 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 a 1.5 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 30Hz for 1-2 minutes.

1. Collect ground plant tissue in a 1.5 ml centrifuge tube. Immediately add 400µl Buffer SP1 and 2µl RNase A. Incubate at 65°C for 10 minutes. Mix sample several times during incubation by inverting tube. **Make 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 140µl Buffer SP2 and vortex to mix. Centrifuge at 12,000 x g for 10 min to pellet cell debris.
3. Carefully aspirate 400 supematant to a 1.5 ml centrifuge tube, making sure not to disturb the pellet or transfer any debris.
4. Add 800µlof MagBead Solution S and 2µl MagBead<sup>®</sup> Particle Solution X

directly into the cleared lysate and mix immediately by vortexing.

**Note: 1.** Dilute MagBead Solution S with absolute ethanol before use. See Page 3 or bottle label for instructions. **2.** MagBeads® Particle Solution X will bead together in its container after several hours. It must be fully suspended by shaking or vortexing before use. **(IMPORTANT)**

5. Incubate the sample at room temperature for 5 minutes.
6. Place the tube on a magnetic separation device suitable for 1.5 ml tube to magnetize the MagBead® particles .
7. Remove and discard the cleared supernatant.
8. Remove the tube containing the Magbead™ particles from the magnetic separation device. Add 500µl of MGW Wash Buffer diluted with ethanol into the tube.
9. Resuspend Magbead® particles pellet by vortexing. Incubate 3 minutes at room temperature. Repeating the mix by vortexing for 1 minutes.
10. Place the plate onto a magnetic separation device to magnetize the Magbead™ particles.
11. Remove and discard the cleared supernatant.
12. Remove the tube containing the Magbead™ particles from the magnetic separation device. Add 500µl of MGW Wash Buffer diluted with ethanol into the tube.
13. Resuspend Magbead™ particles pellet by vortexing.
14. Place the plate onto a magnetic separation device to magnetize the Magbead™ particles.
15. Remove and discard the cleared supernatant.
16. Leave the tube to air dry on the magnetic separation device for 5 minutes. Remove any residue liquid from tube by pipetting.
17. Remove the tube from magnetic separation device. Add 50-100ul Elution Buffer or water to elute DNA from magnetic particles.
18. Resuspend Magbead™ particles by Vortexing. Incubate 5-10 minutes at room temperature. Repeating the mix by vortexing for 1 minutes.
19. Place the tube onto a magnetic separation device to magnetize the Magbead™ particles.

20. Transfer the cleared supernatant containing purified 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<sub>260</sub>)/(absorbance<sub>280</sub>) 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 the MagBead® particle during operation	Carefully avoid remove the MagBead® particles during aspiration
	DNA remains bound to Magbead 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 MagBead particle before elution.

## Ordering Information

Product No.	Product Name	Description
Plant DNA and Plant RNA Isolation Kits		
D3485-01/02 D3486-01/02	Plant DNA Miniprep Kit	Isolation of total cellular DNA from dry and wet plant samples
D3487-01/02	Plant DNA Midiprep Kit	Isolation of total cellular DNA from up to 500 mg plant samples
D3488-01/02	Plant DNA Maxiprep Kit	Isolation of total cellular DNA from up to 2 gram dry and wet plant samples
R6627-01/02 R6827-01/02	Plant RNA Kit	Isolate total cellular RNA from plant samples
R6628-01/02	Plant RNA Midiprep Kit	Isolate up to 800ug total cellular RNA from 800plant samples
Fungal DNA and Fungal RNA Kit		
D3490-01/02 D3390-01/02	Fungal DNA Miniprep Kit	Isolation of total cellular DNA from dry and wet fungal samples
D3590-01/02	Fungal DNA Midiprep Kit	Isolation of total cellular DNA from up to 500 mg fungal samples
R6640-01/02 R6840-01/02	Fungal RNA Kit	Isolate total cellular RNA from fungal samples