

## Contents

Introduction .....	2
Overview .....	2
Storage and Stability .....	2
Kit Contents .....	3
Before Starting .....	3
Section I: Sample preparing .....	4
A. Dry Specimens .....	4
B. Fresh/ Frozen Specimens .....	6
Section II: Vacuum Manifold Processing .....	7
Section III: Centrifugation Processing .....	8
Section IV: Quick Protocol for PCR .....	10
Troubleshooting .....	11
Ordering Information .....	12

**Revised August 2002**

## Introduction

E-Z 96™ Plant DNA Kits allow rapid and reliable isolation of high-quality total cellular DNA from a wide variety of plant species and tissues in a 96-well plate. Up to 30 mg of wet tissue (or 10 mg dry tissue) can be processed in each well in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the HiBind™ matrix with the speed and versatility of the E-Z 96™ DNA plate to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. 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 up to 96 samples to be processed at one time.

## Overview

If using the E-Z 96™ 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. Proteins, polysaccharides, and cellular debris are subsequently precipitated. Contaminants are further removed by isopropanol precipitation of DNA. Binding conditions are then adjusted and the sample is applied to an E-Z 96™ DNA plate. 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

All components of the E-Z 96™ Plant DNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer P3. 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	D1086-01	D1086-02
E-Z 96™ DNA Plate	1	4
96-well Collection Plate (300 µl)	1	4
96-well Collection Plate (2 ml)**	1	4
Adhesive Film Covers	3	12
Buffer P1	60 ml	200 ml
Buffer P2	10 ml	40 ml
Buffer P3	20 ml	80 ml
DNA Wash Buffer Concentrate	48 ml	3 x 60 ml
Instruction Booklet	1	1

\*\* The 96-well plate (2ml) supplied in this kit is for flow-through collection purposes only. The protocol may require an additional 96-well tube rack or 96-well plate (2ml) during sample processing. Both the 96-well tube rack and the 96-well plate (2ml) can be ordered separately from Omega Bio-Tek.

### Before Starting

- Please read the entire booklet to become familiar with the E-Z 96™ Plant DNA Kit procedures.
- Prepare an RNase stock solution at 20 mg/ml and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use. Each sample will require 20 µl of this solution.
- Dilute Wash Buffer Concentrate with ethanol as follows and **store at room temperature**.

**D1086-01**, 1 x 96 preps      Add 72 ml absolute (96%-100%) ethanol to each bottle.

**D1086-02**, 5 x 96 preps      Add 90 ml absolute (96%-100%) ethanol to each bottle.

For bulk packs, refer to Wash Buffer bottle label for dilution instructions.

- Choose the most appropriate protocol to follow. Procedures are described for dried and fresh (or frozen) specimens. In addition, a short protocol is given for isolation of DNA for PCR reactions.

**A. Dry Specimens**      For processing ~10 mg powdered tissue in each well. Yield can be up to 50 µg, depending on the species of plant.  
(page 4)

**B. Fresh/Frozen Specimens**      For processing ≤30 mg fresh (or frozen) tissue. Yield is similar to A.  
(page 6)

**C. Quick Protocol for PCR**  
(page 10)

Rapid protocol for dried or fresh samples by using centrifugation. Yield is sufficient for PCR.

## E-Z 96 Plant DNA Protocol

Materials to be provided by user:

- Microcentrifuge capable of at least 10,000 x g
- Vacuum manifold (for vacuum manifold processing).
- Laboratory centrifuge equipped with swinging-bucket rotor (for centrifugation protocol).
- Adapter for deepwell microplate (for centrifugation processing)
- 96-well plates (96-well deep well plates)
- Nuclease-free 1.5 ml or 2 ml microfuge tubes
- Waterbath equilibrated to 65°C
- Equilibrate sterile dH<sub>2</sub>O water or 10 mM Tris pH 9.0 at 65°C.
- 2-mercaptoethanol
- Isopropyl alcohol (isopropanol)
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/ml
- Liquid nitrogen for freezing/disrupting samples (for fresh/frozen specimens)

## Section I: Sample preparation:

### A. Dry Specimens.

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. To prepare dried samples, place ~10 mg of dried tissue into a microfuge tube and grind using a pellet pestle. Disposable Kontes pestles work well and are available (Cat# SSI-1015-39 & SSI-1014-39). Addition of a pinch of white quartz sand, -50 to 70 mesh (Sigma Chemical Co. Cat No. S9887) will help. For critical work such as PCR and cloning, pestles are best used a single time. Crucibles may be 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 wiping the surface clean between samples. A fine powder will ensure optimal DNA extraction and yield. Process in sets of four to six tubes until step 2 before starting another set. The samples can also be processed in suitable 96 well plates with a mechanical mixer.

1. **To 10 mg powdered dry tissue add 400 µl Buffer P1.** Add 10 µl 2-mercaptoethanol and vortex vigorously to mix. Make sure to disperse all clumps.

**TIP:** Process in sets of four to six tubes: grind, add Buffer P1 and 2-mercaptoethanol, and proceed to step 2 before starting another set. Do not exceed 50 mg dried tissue.

2. **Incubate at 65°C for 10 min.** Mix sample twice during incubation by

inverting the tube or shaking the plate.

- 3. Add 140  $\mu$ l Buffer P2 and vortex to mix.** Centrifuge at  $\geq 10,000 \times g$  for 10 min. (If 96-well tube rack or 2 ml plate is used, centrifuge at  $4000 \times g$  for 20 minutes to pellet the precipitate.)
- 4. Carefully aspirate supernatant to a new microfuge tube** (or 96-well tube rack or 2 ml plate), making sure not to disturb the pellet or transfer any debris. **Add 0.7 volume isopropanol** to the supernatant and vortex to mix the sample and precipitate DNA. This step will remove much of the polysaccharides and improves spin-column performance by increasing DNA binding capacity (and hence yield) in the steps that follow. No incubation is required after addition of isopropanol.

**TIP:** In most cases 350  $\mu$ l supernatant can easily be removed. This will require 245  $\mu$ l isopropanol (0.7 volume). Note that depending on the sample, the volume of supernatant may vary. After transferring to a fresh tube, measure the volume and add the correct amount of isopropanol.

- 5. Immediately centrifuge at 10,000  $\times g$  for 5 min to pellet DNA.** Longer centrifugation does not improve yields. **(If 96-well tube rack or 2 ml plate is used, centrifuge at 4000  $\times g$  for 20 minutes to pellet the DNA.)**
- 6. Carefully aspirate or decant the supernatant and discard, making sure not to lose the DNA pellet.** Invert the microfuge tube (or tube rack or plate) on a paper towel for 1 min to allow residual liquid to drain. It is not necessary to dry the DNA pellet.
- 7. Add 150  $\mu$ l of sterile deionized water, pre-heated to 65° C, and vortex to resuspend the pellet.** A brief incubation at 65° C may be necessary to effectively dissolve the DNA. Add 10  $\mu$ l RNase (20 mg/ml) and mix. No additional incubation is required for RNase treatment.
- 8. Adjust binding conditions of the sample by adding 75  $\mu$ l Buffer P3,** followed by 150  $\mu$ l absolute ethanol and vortex to obtain a homogeneous mixture. A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.
- 9. Apply the entire sample (including any precipitate that may have formed) to the E-Z 96™ DNA plate** by following the procedure described in Section II (Vacuum Manifold Processing) or Section III (Centrifugation Protocol).

## B. Fresh/Frozen Specimens

**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  $\leq 50$  mg. Best results are obtained with young leaves or needles. The method isolates sufficient DNA for several tracks on a standard Southern assay.

To prepare samples, collect tissue in 1.5 ml centrifuge tubes or 96-well tube rack or 2 ml plate and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable Kontes pellet pestles, which are available from Omega Bio-Tek (Cat# SSI-1014-39 & SSI-1015-39). Alternatively, one can allow liquid nitrogen to evaporate and then store samples at  $-70^{\circ}\text{C}$  for later use. For critical work such as PCR and cloning, pestles are best used a single time. Crucibles may be 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.

- 1. Collect ground plant tissue (start with 30 mg) in a microfuge tube (or 96-well tube rack or 2ml plate) and immediately add 300  $\mu$ l Buffer P1.** Add 10  $\mu$ l 2-mercaptoethanol and vortex vigorously. Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.

**TIP:** Process in sets of four to six tubes: fill all tubes with liquid nitrogen, grind, add Buffer P1 and 2-mercaptoethanol, and proceed to step 2 before starting another set. As a starting point, use 20 mg tissue per tube, and if yield and purity are satisfactory increase to 60 mg.

- 2. Incubate at 65°C for 10 min.** Mix sample twice during incubation by inverting tube or shaking the plate.
- 3. Add 110  $\mu$ l Buffer P2 and vortex to mix.** Centrifuge at  $\geq 10,000 \times g$  for 10 min. (If 96-well tube rack or 2ml plate is used, centrifuge at  $4000 \times g$  for 20 minutes to pellet the precipitate.)
- 4. Carefully aspirate cleared lysate to a new microfuge tube (or 96-well tube rack or 2 ml plate) making sure not to disturb the pellet or transfer any debris. Add 0.7 volume isopropanol to the supernatant and vortex to precipitate DNA.** This step will remove much of the polysaccharides and improves spin-column performance by increasing DNA binding capacity (and hence yield) in the steps that follow. No incubation is required after addition of isopropanol.

**TIP:** In most cases 300  $\mu$ l supernatant can easily be removed. This will require 210  $\mu$ l isopropanol (0.7 volume). Note that depending on the sample, the volume of supernatant may vary. After transferring to a fresh tube, measure the volume and add the correct amount of isopropanol.

5. **Immediately centrifuge at 10,000 x g for 5 min to pellet DNA.** Longer centrifugation does not improve yields. (If 96-well tube rack or 2 ml plate is used, centrifuge at 4000 x g for 20 minutes to pellet the precipitate).
6. **Carefully aspirate or decant the supernatant and make sure not to lose the DNA pellet.** Invert the microfuge tube or 96-well tube rack or 2 ml plate on a paper towel for 1 min to allow residual liquid to drain. It is not necessary to dry the DNA pellet.
7. **Add 150 µl of sterile deionized water, pre-heated to 65°C, and vortex to resuspend the pellet.** A brief incubation at 65°C may be necessary to effectively dissolve the DNA. Add 10 µl RNase (20 mg/ml) and mix. No additional incubation is required for RNase treatment.
8. **Adjust binding conditions of the sample by adding 75 µl Buffer P3, followed by 150 µl absolute ethanol, and vortex to obtain a homogeneous mixture.** A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.
9. **Apply the entire sample (including any precipitate that may have formed) to the E-Z 96™ DNA plate** by following the procedure described in Section II (Vacuum Manifold Processing) or Section III (Centrifugation Protocol).

### Section II: Vacuum Manifold Processing

Note: The following protocol is based on using OBI's vacuum manifold (Product No. VAC-03), it may be applied to other manifolds.

1. **Set up vacuum manifold by following manufacturer's instructions.**
2. **Place one 2 ml 96-well collection plate inside the vacuum manifold and place the E-Z 96™ DNA plate on top of the vacuum manifold.**
3. **Apply the entire sample (including any precipitate that may have formed) to the E-Z 96™ DNA plate.**

**Note:** it is always a good idea to mark the E-Z 96™ DNA plate and collection plate at this stage so that they can be easily identified throughout the protocol.

*\*Do not touch the rim of the wells with pipet tips; doing so might result in cross-contamination.*

4. **Turn on the vacuum manifold and filter through the sample mixture by vacuum. Discard the pass through liquid and reuse the 2 ml 96 well collection plate.**
5. **Reassemble the E-Z 96™ DNA plate and the 2 ml 96 well collection plate in the vacuum manifold.**

6. **Add 500 µl DNA wash buffer into each well of the E-Z 96™ DNA plate by using a multichannel pipet.** Wash the plate by turning on vacuum and allowing wash buffer to pass through. (Dilute the DNA wash buffer with ETOH before use.) Switch off the vacuum when pass-through is complete.
7. **Wash the plate again by pipetting 400 µl 100% ethanol into each well of the plate.** Switch on the vacuum until the E-Z 96™ DNA plate is completely dried.
8. **Remove the E-Z 96™ DNA plate from the manifold and tap hard on a stack of paper towels to remove any residual ethanol.** Discard the flow through and collection plate.

**Note:** it is *Very Important* to completely dry the E-Z 96™ DNA plate before elution. Incubate the plate at 70°C for 15 min in an incubator or oven to completely dry the membrane. If a swing bucket centrifuge and 96-well plate adaptor are available, centrifuge at 5000 rpm for 5 minutes to dry the plate.

9. **Place the 300 µl collection plate supplied with the kit inside the vacuum manifold.** If Omega VAC-03 is used, a 800 µl plate is required to be placed under the 300 µl plate to give proper height for elution.

**Note:** If VAC-03 is used, keep the 800 µl plate after experiment for future vacuum processing. A 800 µl 96 well plate is supplied with the VAC-03.

10. **Place the E-Z 96™ DNA plate onto the vacuum manifold.**
11. **To elute the DNA, add 100 ul of preheated (70°C) water or TE buffer to each well of the E-Z 96™ DNA plate using a multichannel pipet. Incubate for 5 min at room temperature and apply the vacuum to elute the DNA into the collection plate.**

**TIP:** 100 ul water or TE buffer is sufficient to elute up to 85% of the DNA from each well of the E-Z 96™ DNA plate. A second elution step with the same 100 ul elutate containing DNA, reheated to 70°C, will increase yield by up to 10-15%.

Total DNA yields vary depending on type and quantity of sample. Typically, 5-50 µg DNA with a  $A_{260}/A_{280}$  ratio of 1.7-1.9 can be isolated using 10 mg dried tissue.

### Section III: Centrifugation Processing

1. **Transfer the entire sample from step 8 of Section I into the E-Z 96™ DNA plate. Use a register chart to identify the position of the samples.**  
**Note:** Add samples to the plate by touching the inside of the wells without moistening the rim.
2. **Place the E-Z 96™ DNA plate onto the 2 ml 96 well collection plate. Seal the top of the DNA plate with an adhesive tape film. Seal the connection of the two plates by using plastic tape if necessary.**
3. **Assemble the DNA plate and the collection plate into a centrifuge adapter for deep-well plate and then place it in the rotor of the centrifuge.**
4. **Centrifuge at 5000 rpm for 5 min.**
5. **Remove the collection plate and discard the flow-through liquid. Reuse the collection plate.**
6. **Remove the tape pad and carefully add 500 µl of DNA Wash Buffer to each well.**
7. **Seal the E-Z 96™ DNA plate with new adhesive film.**
8. **Reassemble the E-Z 96™ DNA plate with the collection plate. Centrifuge at 5000 rpm for 5 min.**
9. **Wash the E-Z 96™ DNA plate with another 500 µl of DNA Wash Buffer by repeating steps 6-8 . Discard the flow-through liquid in the collection plate, reuse the collection plate for next step.**
10. **Add 400 µl absolute ethanol (96-100%) to each well. Seal the E-Z 96™ DNA plate with adhesive film, and centrifuge at 5000 rpm for 5 min.**
11. **Remove the adhesive film, incubate the E-Z 96™ DNA plate at 70°C for 10-15 mins in an incubator or oven to dry the membrane.**

**Note:** Drying the membrane in this step is *Very Important* for DNA elution in the next step. The residue of the DNA Wash Buffer contains ethanol which will inhibit PCR and cause low yield of DNA.

12. **To elute the DNA, place the E-Z 96™ DNA plate on top of the 300µl 96-well collection plate provided and add 100 ul of preheated (70°C) water or TE buffer to each well using a multichannel pipet. Seal the E-Z 96™ DNA plate with new adhesive film and incubate for 2 min at room temperature. Centrifuge at 5000 rpm for 5 min.**

**Tip:** 100 ul water or TE buffer is sufficient to elute up to 85% of the DNA from each well of the E-Z 96™ DNA plate. A second elution step with the same 100 ul eluate containing DNA, reheated to 70°C, will increase yield by up to 10-15%.

Yields vary according to sample size and whether dried or fresh. From 2 µg-50 µg restrictable DNA can usually be obtained with this method.

### Section IV: Quick Protocol For PCR

**Note:** This simplified method allows rapid isolation of DNA from fresh, frozen, or dried specimens for use in PCR reactions. This quick procedure limits the amount of starting material, and DNA yields will generally be lower than (and not as clean as) those obtained with Protocols A and B in section I.

#### Materials to be provided by user:

- 2 ml 96-well plate (Part# SSI-1810-00, sold separately).
- Microcentrifuge capable of at least 5,000 x rpm
- Laboratory centrifuge equipped with swinging-bucket rotor (for centrifugation protocol).
- Adapter for deepwell microplate (for centrifugation processing)
- Nuclease-free 1.5 ml or 2 ml microfuge tubes
- Waterbath equilibrated to 65°C
- Equilibrate sterile dH<sub>2</sub>O water or 10 mM Tris pH 9.0 at 65°C.
- Liquid nitrogen for freezing/disrupting samples (For fresh/Frozen specimens)
- Isopropyl alcohol (isopropanol)
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/ml

Follow suggestions for preparation of dried or fresh samples as outlined in Section I.

1. Note the following limitations on sample size:

- **Dry Samples - use a maximum of 3 mg ground tissue**
- **Fresh Samples - use a maximum of 10 mg fresh/frozen ground tissue**
- 1. **Follow the protocols in Section I to prepare the dried or fresh samples.**

2. **Add 3 mg ground dried sample or 10 mg fresh/frozen samples to each well of a 2 ml 96-well plate .**

Tip: it is always good idea to mark the plate and use a register (96 well) to identify the positions of the samples

3. **Add 300 µl Buffer P1 to each well of the 2 ml 96-well plate.**
4. **Add 110 µl Buffer P2 to each well of the plate. Seal the plate with adhesive film and shake to mix. Add 10 µl RNase (20 mg/ml) and mix.**
5. **Incubate at 65°C for 10 min. Mix sample twice during incubation by shaking the plate.**
6. **Place the 2 ml 96-well collection plate into a centrifuge adapter and centrifuge at 5000 rpm for 15 min.**
7. **Place the E-Z 96™ HiBind DNA plate on top of a new 96-well plate (not**

supplied).

8. Carefully transfer 250 µl of the clear supernatant to each well of the E-Z 96™ DNA plate using a multiple channel transfer pipet. (Note: Mark the plate to identify the positions of samples).
9. Add 125 µl Buffer P3 into each well followed by addition of 250 µl of ethanol (96-100%). Seal the plate with tape. Mix by pipetting or shaking the plate.
10. Place the E-Z 96™ DNA plate on top of the 2 ml 96 well collection plate (supplied) and assemble them into a centrifuge adapter. Centrifuge at 5000 rpm for 5 min.
11. Finish the rest of procedure by following steps 5-12 in Section III.

### Trouble shooting Guide

Problem	Cause	Suggestions
Clogged well	Carry-over of debris.	Following precipitation with Buffer P2, make sure no particulate material is transferred.
	DNA pellet not completely dissolved before applying sample to column.	In protocols A and B, ensure that DNA is dissolved in water before adding Buffer P3 and ethanol. This may need repeated incubation at 65°C and vortexing.
	Sample too viscous.	Do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers P1 and P2 and use two or more columns per sample.
	Incomplete precipitation following addition of P2.	Increase RCF or time of centrifugation after addition of buffer P2.
Low DNA yield	Incomplete disruption of starting material	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer P1.
	Poor lysis of tissue	Decrease amount of starting material or increase amount of Buffers P1 and P2.

	DNA remains bound to column	Increase elution volume to 200 µl and incubate on column at 65°C for 5 min before centrifugation.
	DNA washed off	Dilute Wash Buffer Concentrate 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	Following the second wash spin, ensure that the column is dried by centrifuging 2 min at maximum speed.

### Ordering Information

Product	Description
<b>Plant and Fungal DNA</b>	
D3486-01	E.Z.N.A.™ Plant DNA Miniprep Kit, 50 isolations
D3486-02	E.Z.N.A.™ Plant DNA Miniprep Kit, 200 isolations
D3487-01	E.Z.N.A.™ Plant DNA Midiprep Kit, 10 isolations
D3487-02	E.Z.N.A.™ Plant DNA Midiprep Kit, 25 isolations
D1086-01	E-Z 96™ Plant DNA kit (96 isolations)
D1086-02	E-Z 96.™ Plant DNA Kit, (5 x 96 isolations)
D3490-01	E.Z.N.A.™ Fungal DNA Miniprep Kit, 50 isolations
D3490-02	E.Z.N.A.™ Fungal DNA Miniprep Kit, 200 isolations

### Ordering and technical support contact information:

800-832-8896 (tele) or 770-931-8400 (tele)  
 888-624-1688 (fax) or 770-931-0230 (fax)  
 e-mail: [info@omegabiotek.com](mailto:info@omegabiotek.com)  
 web address: [www.omegabiotek.com](http://www.omegabiotek.com)