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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 in a 96 well plate. Up to 30 mg of wet sample (or 10 mg dry sample) can be processed in each well in less than 1 hour. The system combines the reversible nucleic acid-binding properties of HiBind® matrix with the speed and versatility of 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 procedure. Dry or fresh Plant sample is disrupted and then lysed in a specially formulated buffer containing a proprietary detergent mixture. 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 SP3. It is possible to dissolve such deposits by warming the solution at 37°C.

Kit Contents

Product Number	D1086-01	D1086-02
Preps	1 x 96	4 x 96
E-Z 96® DNA Plate	1	4
96 well Collection Plate (500µL)	1	4
96 well Collection Plate (2mL)	2	2
Buffer SP1	90 mL	2 x 180 mL
Buffer SP2	35 mL	125 mL
Buffer SP3	60 mL	250 mL
DNA Wash Buffer	50 mL	3 x 50 mL
AeraSeal Sealing Film	3	12
Elution Buffer	100 mL	2 x 200 mL
Instruction Booklet	1	1

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 50 mg/mL and aliquot into adequate portions. Store each aliquot at -20ºC and thaw before use. Each sample will require 10 µL of this solution.
- Dilute Wash Buffer with ethanol as follows and **store at room temperature**.

  D1086-01 Add 200 mL absolute (96%-100%) ethanol to each bottle.

  D1086-02 Add 200 mL absolute (96%-100%) ethanol to each bottle.

- 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 (page 4)	For processing ≤10 mg powdered sample in each well. Yield can be up to 50 µg, depending on the species of Plant.
B. Fresh/Frozen Specimens (page 7)	For processing ≤30 mg fresh (or frozen) tissue. Yield is similar to A.

A. E-Z 96 Plant DNA Protocol

Materials to be provided by user

- Laboratory centrifuge equipped with swinging-bucket rotor (for centrifugation protocol).
- Rotor-adapter for deep-well microplate (for centrifugation processing)
- Waterbath equilibrated to 65ºC
- Equilibrate sterile dH_2O water or 10 mM Tris pH 9.0 at 65ºC.
- Isopropyl alcohol (isopropanol; absolute)
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/mL
- Absorbent paper towels
- Liquid nitrogen for freezing/disrupting samples (For fresh/Frozen specimens)
- Vacuum manifold (for vacuum manifold processing only)

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 45º 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 blocks with a mechanical mixer.

1. **To 10 mg powdered dry sample add 400 µl Buffer SP1.** Vortex vigorously to mix. Make sure to disperse all clumps.**TIP**: Process in sets of four to six tubes: grind, add Buffer SP1, 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 tube or shaking the plate.

3. **Add 140 µL Buffer SP2 and vortex to mix.** Incubate the sample for 10 minutes at -20°C. This step helps to remove the proteins, polysacchrides and other inhibitors.

4. **Centrifuge at 4,000 x g for 20 min.**

   NOTE: ***Optional*** E-Z® 96 Lysate Clearance Plates (Product No. EZ1096C) are available for use with this kit at this step. Optionally, desired volume of supernatant following -20°C incubation in Step 3 can be transferred to the E-Z® 96 Filter Plate to be placed over a 1.2 mL 96-well plate (not supplied) and centrifuged at 3,000-5,000 x g for 5 min.

5. **Carefully aspirate 300 µL supernatant to a 96-well plate (not supplied) making sure not to disturb the pellet or transfer any debris.**

6. **Adjust binding conditions of the sample by adding 150 µL Buffer SP3, followed by 300 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.

7. **Apply the entire sample (including any precipitate that may have formed) to an 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 samples allowing more efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of fungi, sample size should be limited to ≤30 mg.

To prepare samples, collect sample 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 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°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 30 mg ground sample in a microfuge tube (or a 2mL 96-well plate) and immediately add 300 µL Buffer SP1.** Mix throughly by vortexing. Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.

2. **Incubate at 65°C for 10 min. Mix sample twice during incubation by inverting tube or shaking the plate.**

3. **Add 100 µL Buffer SP2 and vortex to mix.** Incubate the sample for 10 minutes at -20°C. This step helps to remove the proteins, polysacchrides and other inhibitors.

4. **Centrifuge at ≥10,000 x g for 10 min. (If 2 mL 96-well plate is used, centrifuge at ≥ 4000 x g for 20 minutes to pellet the precipitate.)**

   NOTE: ***Optional*** E-Z® 96 Lysate Clearance Plates (Product No. EZ1096C) are available for use with this kit at this step. Optionally, desired volume of supernatant following -20°C incubation in Step 5 can be transferred to the

E-Z® 96 Filter Plate to be placed over a 1.2 mL 96-well plate (not supplied) and centrifuged at 3,000-5,000 x g for 5 min.

5. **Carefully aspirate 300 µL supernatant to a 96-well plate (not supplied)** making sure not to disturb the pellet or transfer any debris.

6. **Adjust binding conditions of the sample by adding 150 µL Buffer SP3, followed by 300 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.

7. **Apply the entire sample (including any precipitate that may have formed) to an 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).

1. **Set up vacuum manifold by following manufacturer's instructions.**

2. **Place waste collection tray inside the vacuum manifold, then place the E-Z 96® DNA plate on top part of the manifold.**

3. **Apply the entire sample (including any precipitate that may have formed) to an E-Z 96® DNA plate.**

4. **Turn on the vacuum manifold and filter through the sample mixture by vacuum. Turn off the vacuum.**

5. **Add 700 µL DNA wash buffer into each well of the E-Z 96™ DNA plate by using multichannel pipet. Turning on vacuum until all the liquid through the plate. (Dilute the DNA wash buffer with ETOH before use.)**

6. **Wash the plate with another 700 µL DNA wash buffer by repeating step 5.**

7. **Repeat Step 6 by washing the plate with 400 µL 100% ethanol. Continue vacuum until the E-Z 96® DNA plate is completely dried.**

8. **Remove the E-Z 96® DNA plate from manifold and tap hard on a stack of paper towels to remove any residue ethanol. Discard the flow-through and collection plate.**

   Note: It is very important to completely dry the E-Z 96® DNA plate before elution. If a swing bucket centrifuge and 96-well plate adaptor are available, centrifuge at 4,000 x g for 10 minutes to dry the plate.

9. **Assemble the manifold by placing a new 300 µL collection plate (supplied) inside the vacuum manifold**. If an Omega VAC-03 is used, one 800 µL plate should be place under the 300 µL plate to give proper height for elution.

10. **Place the E-Z 96® DNA plate atop the vacuum manifold.**

11. **To elute the DNA, add 100 ul of preheated (65°C) Elution Buffer to each well using a multichannel pipet. Incubate for 5 min at room temperature. Apply the vacuum to elute the DNA into 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 same 100 ul elutate containing DNA, reheated to 65°C, will increase yield by up to 10-15%.

    Total DNA yields vary depending on type and amount of sample. Typically, 5-10 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 6 of Section I into the E-Z 96® DNA plate. Seal the top of the DNA plate with adhesive plate film. Use a register chart to identify the positions of the samples.**

   Note: Do not touch the rims of the wells with pipet tips to avoid cross-contamination.

2. **Place the E-Z 96® DNA plate atop a 2 mL 96 well collection plate (supplied). Connect the two plates by using plastic tape if necessary.**

3. **Place the plates into the centrifuge and spin at 4,000 x g for 5 min.**

4. **Separate the plates and discard the flow-through liquid.** Re-use the collection plate.

5. Remove the adhesive plate film and carefully add 700 ul of DNA wash Buffer to each well of the E-Z 96® DNA plate.

6. **Seal the E-Z 96® DNA plate with new adhesive plate film.**

7. Reassemble the E-Z 96® DNA plate with the collection plate. Centrifuge at 4,000 x g for 5 min.

8. **Separate the plates and discard the flow-through liquid. Wash the DNA plate again with another 700 µL DNA wash Buffer by repeating step 5-7.**

9. Remove the adhesive film, discard the flow-through and place the E-Z 96® DNA plate atop the 300 µL collection plate (supplied). Centrifuge at 4,000 x g for 10 minutes.

   Note: Drying the membrane at this step is very important for DNA elution in next step. The residue of the DNA wash buffer contains ethanol which will inhibit PCR and cause low yield of DNA.

10. **To elute the DNA, add 100 ul of preheated (65°C) Elution Buffer to each well using a mutichannel pipet. Seal the E-Z 96® DNA plate with**

new adhesive film and incubate for 5 min at room temperature. Centrifuge at 4,000 x g for 5 min.

100 ul Elution 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 same 100 ul elute containing DNA, reheated to 65°C, will increase yield by up to 10-15%

Troubleshooting Guide

Problem	Cause	Suggestions
Clogged well	Carry-over of debris.	Following precipitation with Buffer SP2, 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 SP3 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 SP1 and SP2 proportionally.
	Incomplete precipitation following addition of SP2.	Increase RCF or time of centrifugation after addition of buffer SP2.
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
	DNA remains bound to column.	Increase elution volume to 200 µL and incubate 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 step, ensure that the plate is dried by centrifuging 10 min at 4000 x g.