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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 and tissues in a 96 well plate format. E-Z 96® Plant DNA Kits adapted buffer system from OBI's SP Plant DNA system which is particularly useful to process varieties of plants, such as those with unusually high levels of phenolic compounds or polysaccharides: cotton, pine and peanut samples, for example. Up to 50 mg of wet tissue (or 12 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 containing a proprietary detergent mixture. Proteins, polysaccharides, and cellular debris are subsequently precipitated. Binding conditions are then adjusted and the sample is applied to the 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. *Optional* E-Z® 96 Lysate Clearance Plates (Product No. EZ1096C) are available for use with this kit.

New in This Edition

New Equilibration Buffer is introduced into this kit to increase DNA yield and better consistency.

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 SP 1 and SP3. It is possible to dissolve such deposits by warming the solution at 37°C.

Kit Contents

Product Number	1086-01	D1086-02
E-Z 96 [®] DNA Plate	1	4
Round Well Plate (1.2ml)	1	4
Caps for Round well Plate	24 x 8	96 x 8
Racked Microtubes (1.2 mL)	2	8
8-Strip Microtube Caps	36 x 8	144 x 8
AeraSeal Film	4	16
Deep Well Collection Plate (2 ml)	1*	2*
Buffer SP1	60 ml	240 ml
Buffer SP2	20 ml	80 ml
Buffer SP3	30 ml	100 ml
SPW Wash Buffer	40 ml	160 ml
Equilibration Buffer	18 ml	70 mL
Elution Buffer	50 ml	150 ml
RNase A	450µl	1.35 ml
Instruction Booklet	1	1

*2 ml plates are reusable. See below for cleaning instructions.

Before Starting

Please read this manual to become familiar with all E-Z 96[®] Plant DNA Kit procedures.

- Equilibrate Buffer SP1 and DNA Elution Buffer to 65° C (SP1 should be equilibrated to 80° C if liquid nitrogen is used for sample disruption).
- Prepare the SP1/RNase A stock solution: Add 2 µl RNase A for each 400 µl SP1 Buffer. (One vial RNase A should be added to one bottle Buffer SP1.)
- Dilute **SP3 Buffer** with **absolute ethanol** as follows

D1086-01	Add 60 ml absolute (96%-100%) ethanol to bottle.
D1086-02	Add 200 ml absolute (96%-100%) ethanol to each bottle.

- Dilute **SPW Wash Buffer** with **absolute ethanol** as follows

D1086-01	Add 160 ml absolute (96%-100%) ethanol to bottle.
D1086-02	Add 640 ml absolute (96%-100%) ethanol to each bottle.

- 2ml deep well plates can be used to collect flow-through from the E-Z 96[®] DNA plate. They are designed for repeated use. Wash the plates thoroughly in tap water after each use. Incubate 5 minutes at room temperature in 0.5M Hcl. Rinse with distilled water. Used plates can also be autoclaved after washing.

E-Z 96[®] Plant DNA Protocol

Equipment supplied by user

- Laboratory centrifuge equipped with swing-bucket rotor at room temperature capable of at least 5,000 x g. (For centrifugation protocol)
- Waterbath equilibrated to 65° C
- Oven or incubator preset at 65° C
- Absolute (96%-100%) ethanol
- Liquid nitrogen for freezing/disrupting samples (For Fresh/Frozen Specimens)
- Equipment for disrupting plant tissue.
- Ice, freezer or 96-well cryorack at -20° C

Disruption of Plant Tissues

Grind sample with pestles

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 ~15 mg of dried tissues into a microfuge tube (1.5 ml tubes are recommended) and grind using a pellet pestle. Disposable Kontes pestles work well and are available from Omega Bio-Tek (Cat# SSI-1014-39 & SSI-1015-39). 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. A fine powder will ensure optimal DNA extraction and yield.

B. Fresh/Frozen Specimens

Due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to ≤50 mg from beginning. It is very important not overload the E-Z 96[®] DNA plate. Too much starting material will decrease the yield and purity due to the inefficient lysis. However, for some plant species increasing the starting material can increase DNA yield. We recommend starting with 50 mg tissue at first. If results obtained are satisfactory, then increase amount of starting material to optimal level. Best results are obtained with young leaves or needles.

Various means of sample disruption can be used for this kit, such as beads, pestles, etc., but use of liquid nitrogen is recommended with each. One example: 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 Kontes pellet pestles, which are available from OBI (Cat# 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 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. **Transfer the ground sample into a 96-well tube rack or deep well plate.**

Note: Do not allow the sample to thaw during handling and weighing. To prevent sample from thawing, keep the rack or plate on a bed of dry ice.

Disrupting Samples With Beads Mill

Fresh or dried plant tissue samples can be effectively disrupted and homogenized by rapid agitation in the presence of beads. Leaf samples and beads are added to each well of a 96-well tube rack or deep well plate. The racks or plates are fixed into clamps on a mixer mill. Tissue samples are disrupted and simultaneously homogenized with the shearing and crushing action of the beads.

1. After disrupting sample material, measure up to 15 mg dry powdered tissue or 50mg fresh (or frozen) tissue and place into 1.2 ml Round Well Plate(supplied). Add 400 µl SP1/RNase A solution to each sample. Seal the wells with Caps for Round well Plate (supplied).

Note: Upper limits of 50 mg (wet weight) or 15 mg (dry weight) of starting material are recommended. More or less can be used depending on results. Water content (and buffer absorption) of samples affect optimum starting amounts. Suspend the samples thoroughly by vortexing or shaking (**side to side**). Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.

2. Incubate at 65°C for 10 min. Mix samples twice during incubation by briefly shaking the plate (side to side).
3. Remove and discard Caps for Round well Plate. Add 140 µl Buffer SP2 to each lysate.
4. Close the wells with new Caps for Round well Plate(supplied). Vortex or shake the rack **side to side** somewhat vigorously for 20 seconds. Centrifuge the round well plate briefly(<1 min) to collect any liquid from the caps. Do not prolong this step.

It is critical to mix the sample throughly by shaking vigorously to give optimized yield. The brief centrifugation prevent the precipitates from freezing to the cap, which otherwise could be difficult to open the cap after incubation at -20°C in next step.

5. Incubate the round well plate for 10 minutes at -20°C. This step helps to remove the proteins, polysaccharide and other inhibitors.
6. Centrifuge the round well plate for 10 minutes at 3,000-5,000 x g. Compact pellets will form in the tubes but some particles may float. **Be careful to avoid those particles during the transfer in next step.**

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 96-tube rack and centrifuged at 3,000-5,000 x g for 5 min. Following centrifugation the protocol would continue at Step 8.

7. Remove the caps and carefully transfer 400 µl of each supernatant to the Racked Microtubes (1.2 mL). If less than 400 µl of supernatant is recovered, adjust volume of SP3/ethanol in Step 8.
8. Add 1.5 volume (600µl for 400 µl lysate) of SP3/ethanol solution. A precipitate may form upon addition of SP3/ethanol; it will not interfere with DNA isolation.
9. Close the microtubes with 8-Strip Microtube Caps (supplied) and vortex or shake the tube rack side to side somewhat vigorously for 20 seconds. Centrifuge the racked microtubes briefly to collect any liquid from the caps. Allow the centrifuge to reach 3000 rpm, and stop the centrifuge. Do not prolong this step.
10. Place the HiBind[®] DNA Plate on top of the 2ml collection plate (supplied). Add 150µl Equilibration Buffer into each well and let the plate sit for 4 minutes at room temperature. Centrifuge at 3000-5000 x g for 2 minutes.
11. Remove and discard the 8-Strip Microtube Caps . Carefully transfer 1 ml of each sample into the HiBind[®] DNA Plate. Be careful not to spill sample liquid onto the rims of the wells during the transfer.
12. Seal the HiBind[®] DNA Plate with AeraSeal film (supplied). Centrifuge at 3,000-5,000 x g for 5 minutes or until all the sample passes through the HiBind[®] membrane.
13. Remove the AeraSeal film and discard the flow-through from the deep well collection plate. Reassemble the HiBind[®] DNA Plate with deep well collection plate.
14. Add 800µl SPW Wash Buffer to each well of the HiBind[®] DNA Plate. Seal the plate with AeraSeal film. Centrifuge at 3,000-5,000 x g for 5 minutes. Remove the AeraSeal film and discard flow-through.
15. Wash the HiBind[®] DNA Plate again with another 800 µl SPW Wash Buffer. Seal with new AeraSeal film. Centrifuge at 5,000 x g for 15 minutes to dry the plate. Drying the plate *completely* is **critical** for removal of residual ethanol that will otherwise interfere with downstream applications.

16. Remove the AeraSeal film. To elute the DNA, place the HiBind® DNA Plate in right orientation on a Racked Microtubes (1.2 mL) (supplied) or a desired 96-well microplate(not supplied). Add 100 µl Elution Buffer (pre-heated at 65°C) to each sample. Seal the plate with a new AeraSeal film (supplied). Incubate for 5 minutes at 65°C in a incubator. Centrifuge at 5,000 x g for 5 minutes.
17. Repeat Step 16 with another 100 µl Elution Buffer (pre-heated at 65°C). To maintain higher DNA concentration, optionally, second elution may be performed with first eluate.
18. Seal the Racked Microtubes(1.2mL) with 8-Strip Microtube Caps (supplied).

Vacuum Manifold Protocol (Optional)

Note: The following protocol is based on using OBI's vacuum manifold (Product No. Vac-03)

1. Assemble the vacuum manifold : 1) Place the E-Z 96® DNA plate on the top part of vacuum manifold. 2) Place the waste collection tray inside base part of the manifold. Seal the unused wells of the E-Z 96® DNA plate with AeraSeal film tape.
2. Grind sample and prepare the cleared lysate by following Steps 1-10.
3. Apply 1 ml of the sample (including any precipitate that may have formed) to the E-Z 96® DNA Plate.

Note: it is always 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 to avoid cross-contamination.**

4. Turn on the vacuum manifold and filter through the sample mixture by vacuum. Turn off the vacuum and load the remainder of the sample into the DNA Plate (if any). Apply the vacuum to draw the sample through the DNA Plate.
5. Add 800 µl SPW Wash Buffer into each well of the E-Z 96® DNA plate by using a multichannel pipet. Place the plate into vacuum manifold and wash the plate by turning on vacuum. (Dilute the DNA wash buffer with ETOH before use.)
6. Repeat Step 5 with an additional 800 µl SPW Wash Buffer.
7. Wash the plate with 400 µl absolute (96%-100%) ethanol. Continue the 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 ethanol residue. 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 with a 96-well plate adaptor is available, centrifuge at 5000 x g for 5 minutes to dry the plate. Or if a vacuum oven is available, place plate in oven set to 70°C for 10 minutes.

9. Assemble the manifold by placing a new Racked Microtubes (1.2 mL) inside the vacuum manifold.
10. Place the E-Z 96® DNA plate onto vacuum manifold.
11. To elute the DNA, add 100 ul of preheated Elution Buffer to each well using a multichannel pipet. Reassemble the E-Z 96® DNA plate with new collection plate into vacuum manifold and incubate for 5 min at room temperature. Apply the vacuum to elute the DNA into collection plate.
TIP: 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%. Total DNA yields vary depending on type and quantity of sample.
12. Seal the tube with 8-Strip Microtube Caps (supplied)

Troubleshooting Guide

Problem	Cause	Suggestions
Clogged well	Carry-over of debris.	Following precipitation with Buffer SP2, make sure no particulate material is transferred.
	Sample too viscous.	Do not exceed suggested amount of starting material.
	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 on plate membrane at 65°C for 5 min before centrifugation.
	DNA washed off.	Dilute SPW Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (Page 3).
Problems in downstream applications	Salt carry-over.	SPW Buffer must be stored at room temperature.
	Ethanol carry-over	Following the second wash spin, ensure that the plate is completely dried before elution.