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Introduction

E.Z.N.A.™ SP Plant Kit is specially designed for rapid and reliable isolation of high-quality total cellular DNA from plant species that contains high level of phenolic and polysaccharides. Up to 100 mg of wet tissue (or 30 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of HiBind™ matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. The new introduced homogenization column provide a easy and fast tool for sample homogenization process. 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.™ SP Plant 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 detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated. Binding conditions are then adjusted and the sample is applied to a HiBind™ DNA spin-column. 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.N.A.™ SP Plant Kits 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, though we have found that they do not interfere with overall performance.

Kit Contents

Product Number	D5510-00	D5510-01	D5510-02
HiBind™ DNA Columns	5	50	200
2 ml Collection Tubes	10	100	400
Omega Homogenizer Column	5	50	200
Buffer SP1	5 ml	50 ml	180 ml
Buffer SP2	1ml	10 ml	30 ml
Buffer SP3	1.5 ml	10ml	40 ml
SPW Buffer	5 ml	20 ml	3 x 20 ml
Elution Buffer	1.2 ml	15 ml	50 ml
Instruction Booklet	1	1	1

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.™ SP Plant Miniprep Kit procedure.
- Prepare an RNase stock solution at 50 mg/ml and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use. Each sample will require 10 µl of this solution.
- Prepare **SP3/ethanol** stock solution as follows:

D5510-00, Trial Kit	Add 3 ml absolute (96%-100%) ethanol.
D5510-01, 50 preps	Add 20ml absolute (96%-100%) ethanol to each bottle.
D5510-02, 200 preps	Add 80 ml absolute (96%-100%) ethanol to each bottle.

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

D5510-00, Trial Kit	Add 20 ml absolute (96%-100%) ethanol.
D5510-01, 50 preps	Add 80ml absolute (96%-100%) ethanol to each bottle.
D5510-02, 200 preps	Add 80 ml absolute (96%-100%) ethanol to each bottle.

- Choose the most appropriate protocol to follow. Procedures are described for each of 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 ~30 mg powdered tissue. DNA yield vary depending on genome size, ploidy, and age of samples. Yields typically range from 5-50µg for 30 mg dry samples.
B. Fresh/Frozen Specimens (Page 6)	For processing ≤100 mg fresh (or frozen) tissue. Yields typically ranges from 3-30µg.

Plant Miniprep Protocol

A. Dry Specimens

Materials to be provided by user:

- Microcentrifuge capable of at least 10,000 x g
- Nuclease-free 1.5 ml or 2 ml microfuge tubes
- Waterbath equilibrated to 65ºC
- Equilibrate Elution Buffer at 65ºC.
- 2-mercaptoethanol
- Absolute (96%-100%) ethanol
- RNase A stock solution at 50 mg/ml

This is the most robust method for isolation of total cellular (mitochondrial, chloroplast and genomic) DNA. Yields are usually sufficient for several tracks on a Southern blot for RFLP mapping.

Drying allows storage of field specimens for prolonged periods of time prior to processing. Samples can be dried overnight in a 45ºC oven, powdered, and stored dry at room temperature. To prepare dried samples place ~30 mg of dried tissue into a microfuge tube (2 ml tubes are recommended for processing of >50 mg tissue) 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. 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.

1. **To 10-30 mg powdered dry tissue add 600 µl Buffer SP1 following by** adding 10 µl of RNase A stock solution. Mix throughly by votexing. Make sure to disperse all clumps.
   Note: Make sure to disperse all tissue clumps with pipetting or votexing. Clumped tissues will not be lysed properly and will cause lower yield of DNA.

2. **Incubate at 65ºC for 10-20 min. Mix sample few times by inverting the tube during incubation by inverting tube.**

3. **Add 210 µl Buffer SP2 and vortex to mix. Incubate the samples for 5 minutes on ice. Centrifuge at ≥10,000 x g for 10 min.**

4. **Carefully aspirate supernatant to a Omega Homogenizer Column making sure not to disturb the pellet or transfer any debris.**

5. **Immediately centrifuge at 10,000 x g for 2 min.** Longer centrifugation does not improve yields. Omega Homogenizer Column will remove most remaining precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be careful not disturb this pellet in step 6.

6. **Carefully transfer flow-through into a new 1.5ml microtube and making sure not to dislodge the pellet.** Measure the volume of the lysate for next step.

7. **Adjust binding conditions of the sample by adding 1.5 volume of Buffer SP3/ethanol mixture and vortex to obtain a homogeneous mixture.**

8. **Transfer 650 µl supernatant to a HiBind™ DNA column placed in a 2 ml collection tube (supplied) .** Centrifuge the column at 10,000 x g for 1 min to bind DNA. Discard the flow-through liquid and re-use the collection tube for step 9.

   Note: This the point to start the optional vacuum/spin protocol. (See Page 10 for details)

9. **Repeat step 8 with remaining of sample. Discard flow-through and collection tube.**

10. **Place the column into a new collection tube and add 650 µl SPW Buffer.** Centrifuge at 10,000 x g for 1 min and discard the flow-through liquid. Reuse the collection tube in Step 11 below.

    NOTE:SPW Concentrate must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.

11. **Repeat wash step with an additional 650 µl SPW Buffer.** Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse 2 ml collection tube in Step 12.

12. **Centrifuge empty column 2 min at maximum speed to dry.** This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.

13. **Transfer column to a clean 1.5 ml tube. Apply 100 µl Elution Buffer pre-warmed to 65°C and incubate at room temperature for 1 min. Centrifuge at 10,000 x g for 1 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 200 µl of buffer for elution is not recommended.

14. **Repeat Step 13 with an additional 100 µl of buffer.** This may be performed using another 1.5 ml tube to maintain a higher DNA concentration in the first eluate.

TIP: To increase DNA concentration add buffer and incubate the column at 60°C-70°C for 5 min before elution.

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 30 mg dried tissue.

B. Fresh/Frozen Specimens

Materials to be provided by user:

- Microcentrifuge capable of 10,000 x g
- Nuclease-free microfuge tubes
- Water bath equilibrated to 65°C
- Equilibrate Elution Buffer at 65°C.
- 2-mercaptoethanol
- Absolute (96%-100%) ethanol
- Liquid nitrogen for freezing/disrupting samples
- RNase A stock solution at 50 mg/ml

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 ≤100 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 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.

1. **Collect ground plant tissue (start with up to 100 mg) in a microfuge tube and immediately add 400 µl Buffer SP1.** 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.**

3. **Add 140 µl Buffer SP2 and vortex to mix. Centrifuge at ≥10,000 x g for 10 min.**

4. **Carefully aspirate supernatant to a Omega Homogenizer Column making sure not to disturb the pellet or transfer any debris.**

5. **Immediately centrifuge at 10,000 x g for 2 min.** Longer centrifugation does not improve yields. Omega Homogenizer Column will remove most remaining precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be careful not disturb this pellet in step 6.

6. **Carefully transfer flow-through into a new 1.5ml microtube and making sure not to dislodge the pellet.** Measure the volume of the lysate for next step.

7. **Adjust binding conditions of the sample by adding 1.5 volume of Buffer SP3/ethanol mixture and vortex to obtain a homogeneous mixture.**

8. **Transfer 650 µl supernatant to a HiBind™ DNA column placed in a 2 ml collection tube (supplied) .** Centrifuge the column at 10,000 x g for 1 min to bind DNA. Discard the flow-through liquid and re-use the collection tube for step 9.

9. **Repeat step 8 with remaining of sample. Discard flow-through and collection tube.**

   Note: This the point to start the optional vacuum/spin protocol. (See Page 10 for details.)

10. **Place the column into a new collection tube and add 650 µl SPW Buffer.** Centrifuge at 10,000 x g for 1 min and discard the flow-through liquid. Reuse the collection tube in Step 11 below.

    NOTE:SPW Concentrate must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label

11. **Repeat wash step with an additional 650 µl SPW Buffer.** Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse 2 ml collection tube in Step 12.

12. **Centrifuge empty column 2 min at maximum speed to dry.** This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications

13. **Transfer column to a clean 1.5 ml tube. Apply 100 µl Elution Buffer pre-warmed to 65°C and incubate at room temperature for 1 min. Centrifuge at 10,000 x g for 1 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 200 µl of buffer for elution is not recommended.

14. **Repeat Step 13 with an additional 100 µl of Elution buffer.** This may be performed using another 1.5 ml tube to maintain a higher DNA concentration in the first eluate.

    TIP: To increase DNA concentration add buffer and incubate the column at 60°C-70°C for 5 min before elution.

Total DNA yields vary depending on type and quantity of sample. Typically, 3-30 µg DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 100 mg fresh leaf tissue.

Troubleshooting

Problem	Cause	Suggestions
Clogged column	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 SP3 and ethanol. This may need repeated incubation at 65°C and vortexing.
	Sample too viscous.	In Protocol C, do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers SP1 and SP2 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 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 column 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.	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.