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Introduction

The E.Z.N.A.™ family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is the new HiBind® matrix that specifically, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Since phenol/chloroform extractions are not needed time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA obtained using the E.Z.N.A.™ Nucleic Acid purification system can be directly used for a wide range of applications such as PCR, Southern Blotting, and Restriction Enzyme Digestion.

The E.Z.N.A.™ SP Plant DNA Kit provides an easy and rapid method for the isolation of high quality total cellular DNA from plant species containing high levels of phenolic compounds and polysaccharides. Up to 100 mg of wet tissue (or 30mg of dry tissue) can be processed in less than 45 minutes.

If you are using the E.Z.N.A.™ SP Plant DNA Kit for the first time, please make sure that you read this booklet thoroughly and become familiar with the procedures prior to performing experiment. With a specially formulated detergent containing buffer you will begin by disrupting and lysing your dry or fresh plant tissue sample. Proteins, polysaccharides, and any cellular debris will be subsequently precipitated. Binding Conditions will then be adjusted with a buffer/ethanol solution, and the sample will be applied to a HiBind® DNA mini column. Two rapid wash steps will remove any trace contaminants (i.e. polysaccharides, cellular debris), and then pure DNA will be eluted in water or a low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All E.Z.N.A.™ SP Plant DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature (22-25°C). It is possible that during shipment or storage in cool ambient conditions, that a precipitate has formed in Buffers SP1 and SP3. Dissolve such deposits by warming each solution to 37°C with gentle shaking or stirring.

Kit Contents

Product Number	D5511-00	D5511-01	D5511-02
Purification Times	5 preps	50 preps	200 preps
HiBind®DNA Mini Columns	5	50	200
Omega® Homogenizer Columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer SP1	5 ml	40 ml	150 ml
Buffer SP2	1ml	12 ml	50 ml
Equilibration Buffer	1.2 mL	12 mL	45 mL
Buffer SP3 (concentrate) † ▲	2 ml	40 ml	100 ml
RNase A	30 µl	275 µl	1.10 ml
SPW Wash Buffer (concentrate) †	5 ml	20 ml	3 x 20 ml
Elution Buffer *	1.5 ml	15 ml	60 ml
Instruction Booklet	1	1	1

* Elution Buffer = 10mM Tris-HCl, pH 8.5 . Buffer SP3 contains a chaotropic salt, please take special precaution when handling this agent. Buffer SP3 is not compatible with disinfectants containing bleach.

Before Starting

It is strongly advised that you familiarize yourself with the entire booklet before starting. E.Z.N.A ™ Kits are designed to be simple, fast, and reliable provided that all steps are followed diligently.

A. Dry Specimen Protocol (page 5)	Ideal for processing ≤30mg of powdered tissue samples. DNA yields will vary and will depend on genome size, ploidy, and sample age. Yields based on a 30mg sample will range any where from 5 to 50µg.
B. Fresh/Frozen Specimen Protocol (page 9)	Ideal for processing ≤100mg of fresh or frozen tissue. A 100mg specimen sample will typically yield 3-30µg of DNA.

Important	Set waterbath set to 65° C
	Prepare **SP3/ethanol** stock solution as follows **D5511-00** Add 4 ml of absolute **D5511-01** Add 80 ml of absolute ethanol to each bottle **D5511-02** Add 200 ml of absolute ethanol to each bottle
	Dilute **SPW Wash Buffer** with **absolute ethanol** as follows **D5511-00** Add 20 ml of absolute ethanol **D5511-01** Add 80 ml of absolute ethanol **D5511-02** Add 80 ml of absolute ethanol to each bottle

A. E.Z.N.A.™ SP Plant DNA Kit Dry Specimen Protocol

Materials and Reagents to be supplied by user

- Microcentrifuge capable of at least 10,000 x g
- Nuclease-free 1.5 ml or 2 ml microfuge tubes
- Elution Buffer equilibrated to 65°C
- Absolute(~96-100%) Ethanol
- Ice or Cryorack for microcentrifuge tubes
- Liquid Nitrogen (for fresh/frozen samples)

This is the most robust method for the 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 45°C oven, and then powdered and stored at room temperature.

NOTE: Samples should be completely dried within 24 hours of collection to ensure DNA Quality

For the preparation of dried samples place ~ 30 mg of dried tissue into a microfuge tube, and grind using a pellet pestle. *Disposable Kontes pestles work well and are available for purchase (product no. SSI-1014-39 & SSI-1015-39).* For critical work, such as PCR and cloning, pestles are best used when used only a single time and then promptly soaked in a dilute bleach solution 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.

NOTE: Recommended starting amount for dry tissue samples is 10-30 mg. Exceeding this amount will reduce yield and purity.

1. **To 10-30 mg of powdered dry tissue add 600 µl of Buffer SP1, followed by the addition of 5µl of RNase A solution. Mix thoroughly by vortexing vigorously.** Make sure to disperse all clumps.

   NOTE: Disperse all clumps by pipetting or vortexing. Clumped tissue will not lyse properly, and will result in lower DNA yields.
   NOTE: Do not mix SP1 and RNase A before use.
2. Lyse Cells: **Incubate at 65°C for 10 min. Mix sample several times during incubation by inverting tube.**

3. Precipitate Detergent, proteins and polysaccharides: **Add 210 µl of 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 an Omega® Homogenizer Column placed into a 2 ml collection tube** (supplied). Make 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. The Omega® Homogenizer Column will remove most remaining precipitates and cell debris, but a small amount might pass through, and form a pellet in the collection tube. Be careful not to disturb this pellet in Step 6.

6. **CAREFULLY, transfer cleared lysate into a 1.5ml microtube** (not supplied)**, making sure not to dislodge the pellet. Measure the volume of the lysate** (you will need to know for the following step).

7. **Adjust binding conditions of the sample by PIPETTING 1.5 volumes of Buffer SP3/ethanol mixture DIRECTLY onto cleared lysate. Vortex IMMEDIATELY to obtain a homogenous mixture.** If precipitation can be seen at this point, break the precipitation by passing the mixture through a needle using a syringe or by pipetting up and down 10-15 times.
   NOTE: Please Ensure that you have added Absolute ethanol to Buffer SP3 prior to use.

8. Prepare the column by adding 200 µl of Equilibration Buffer placed in a 2 mL collection tube. Centrifuge at ≥ 13,000 x g for 30-60 seconds. Discard the flowthrough and reuse the collection tube.
9. **Transfer 650 µl of the supernatant to a HiBind® DNA Mini Column placed into a 2ml collection tube** (supplied). **Centrifuge the column at 10,000 x g for 1 min to bind DNA. Discard flow-through and re-use the collection tube in step 10.**
10. **Repeat step 9 with if you have any sample remaining. Discard flow-through and collection tube.**

11. **Place the column into a new 2 ml collection tube, and add 650µl of SPW Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min and discard the flow-through.** Re-use the collection tube in the following step.

    NOTE: SPW Wash Buffer is supplied as a concentrate and must be diluted

with absolute (96-100%) ethanol prior to use. Please follow the instructions on the label or refer to page 4 of this manual.

12. **REPEAT wash step by adding another 650µl fo SPW Wash Buffer**. **Centrifuge at 10,000 x g for 1 min. Discard flow-through and re-use the collection tube in Step 13.**

13. **Using the same collection tube, centrifuge the empty column for 2min at maximum speed (≥10,000 x g) to dry.**

    NOTE: This step is critical for removing residual ethanol that might otherwise be eluted in the DNA, and interfere with downstream applications.

14. **Transfer the HiBind® DNA Mini column into a clean 1.5ml tube** (not supplied)**. Apply 50-100µl of Elution Buffer** (volume will depend on the desired final DNA concentration; for higher DNA concentration reduce elution volume) **pre-warmed to 65° and incubate at room temperature for 3-5 min. Centrifuge at 10,000 x g for 1min to elute DNA .** Smaller elution volumes will significantly increase DNA concentration but will provide lower yields.

15. **REPEAT step 14 with an additional 50-100µl of Elution Buffer.** This may be performed using another 1.5 ml tube in order to maintain a higher DNA concentration in the first eluate. Use of more than 200 µl of Elution Buffer is not recommended.
    TIP: To increase DNA yield, add Elution Buffer and incubate the column at 65°C for 5 min before elution. Alternatively, DNA yield and concentration can be increased by using the first eluate for the second elution.

    NOTE: Total DNA yields will vary depending on type and quantity of sample. Typically, 5-50µg of DNA with an A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 30 mg of dried tissue sample.

B. E.Z.N.A.™ SP Plant Mini Protocol for Frozen Specimens

NOTE: USE EXTREME CAUTION WHEN HANDLING LIQUID NITROGEN.

This protocol is suitable for most fresh or frozen tissue samples, allowing efficient DNA recovery. However, due to the tremendous variation in water and polysaccharide content in plants, sample size should be limited to ≤ 100 mg. Best results are obtained with young leaves or needles. This method typically isolates sufficient DNA for several tracks on a standard Southern Blot Assay.

To prepare samples, collect tissue in a 1.5ml or 2ml 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 for purchase (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 when used only a single time and then promptly soaked in a dilute bleach solution 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

NOTE: Recommended starting amount for fresh/frozen samples is 50-100 mg. Exceeding this amount will reduce yield and purity

1. **Collect ground plant tissue in a microfuge tube and IMMEDIATELY add 400µl of Buffer SP1**, **followed by the addition of 5µl of RNase A solution.** Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.
   TIP: Begin with 50 mg of tissue. As results are satisfactory, increase the amount of starting material up to 100mg.

2. Lyse Cells: **Incubate at 65°C for 10 min. Mix sample several times during incubation by inverting tube.**
3. Precipitate Detergent, proteins and polysaccharides: **Add 140 µl of 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 an Omega® Homogenizer Column placed into a 2ml collection tube** (supplied). Make 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. The Omega® Homogenizer Column will remove most remaining precipitates and cell debris, but a small amount might pass through, and form a pellet in the collection tube. Be careful not to disturb this pellet in Step 6.

6. **CAREFULLY, transfer cleared lysate into a 1.5ml microtube** (not supplied)**, making sure not to dislodge the pellet. Measure the volume of the lysate** (you will need to know for the following step).

7. **Adjust binding conditions of the sample by PIPETTING 1.5 volumes of Buffer SP3/ethanol mixture DIRECTLY onto cleared lysate. Vortex IMMEDIATELY to obtain a homogenous mixture.** If precipitation can be seen at this point, break the precipitation by passing the mixture through a needle using a syringe or by pipetting up and down 10-15 times. .

   NOTE: Please Ensure that you have added Absolute ethanol to Buffer SP3 prior to use.

8. **CONTINUE BY FOLLOWING STEPS 8-15 OF THE DRY SPECIMEN PROTOCOL.**

Troubleshooting Guide

Problem	Cause	Suggestions
Clogged column	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.	Ensure that DNA is dissolved in water before adding Buffer SP3 and ethanol. This may need repeated incubation at 65°C and vortexing.
	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 column at 65°C for 5 min before centrifugation.
	DNA washed off.	Dilute SPW Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (Page 4).
Problems in downstream applications	Salt carry-over.	SPW 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.

The Following Products are available for Purchase Separately

Product	Size	Product No.
Buffer SP1	250 ml	PD072
Buffer SP2	60 ml	PD073
Buffer SP3	100 ml	PD074
SPW Wash Buffer Concentrate	25ml	DR045
Omega® Homogenizer Columns	200/bag	HCR003
RNase A	400µl / 5ml	AC117/AC118
Elution Buffer	100ml	DR048
0.5ml Homogenization Pestles (with 0.5ml microfuge tubes)	10/bag	SSI-1014-39
1.5ml Homogenization Pestles (with 1.5ml microfuge tubes)	10/bag	SSI-1015-39
2ml capless collection tubes	500/BAG	SS1-1370-00
1.5ml DNase/RNase Free Centrifuge Tubes	500/BAG	SS1-1210-00

Please call for Pricing and availability:

Tel: 770-931-8400 (US)
Fax: 770-931-0230 (US)
e-mail: info@omegabiotek.com

Tel: 1-800-832-8896 (Toll free)
Fax: 1-888-624-1688 (Toll free)
www.omegabiotek.com