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Introduction

The E.Z.N.A.® SP Plant DNA Maxi Kit is specially designed for rapid and reliable isolation of high-quality total cellular DNA from plant species containing high levels of phenolic compounds and polysaccharides. Up to 1 g of wet tissue (or 250 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the HiBind® matrix with the speed and versatility of spin column technology 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 multiple samples to be processed in parallel.

Overview

If using the E.Z.N.A.® SP Plant DNA Maxi 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. Binding conditions are then adjusted and the sample is applied to a HiBind® DNA maxi 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 DNA Maxi 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 Buffers SP1 and SP3. It is possible to dissolve such deposits by warming the solution at 37°C and gently shaking or stirring.

Binding Capacity

Each HiBind™ DNA Maxi Column can bind up to 1 mg of genomic DNA. Use More than 2 g Fresh plant samples is not recommend.

Kit Contents

Product Number	D5538-00	D5538-01	D5538-02
Purification Times	2 Preps	5 Preps	20 Preps
HiBind® DNA Maxi Columns	2	5	20
Homogenizer Columns	2	5	20
50 ml Collection Tubes*	4	10	40
Buffer SP1	20 ml	40 ml	160 ml
Buffer SP2	6 ml	15 ml	60 ml
Buffer SP3	7 ml	20 ml	75 ml
SPW Buffer Concentrate	5 ml	25 ml	2 x 50 ml
Elution Buffer	5 ml	30 ml	100 ml
Instruction Booklet	1	1	1

Note : 50 ml collection tubes have been inserted with HiBind™ DNA Maxi columns and Homogenizer Columnes.

Materials to be provided by user

- Centrifuge capable of at least 3,000 x g
- Nuclease-free 50 ml high-speed centrifuge tubes
- Waterbath equilibrated to 65°C
- Absolute (96%-100%) ethanol
- RNase A stock solution at 50 mg/ml
- Ice or Cryorack for centrifuge tubes
- (Optional for Fresh sample)Liquid nitrogen for freezing/disrupting samples

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.® SP Plant DNA Maxi Kit procedures.
- 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 20 µl of this solution.
- Equilibrate Elution Buffer and Buffer SP1 at 65°C.
- Prepare **Buffer SP3/ethanol** stock solution as follows:

D5538-00	Add 14 ml absolute (96%-100%) ethanol.
D5538-01	Add 40ml absolute (96%-100%) ethanol to each bottle.
D5538-02	Add 150 ml absolute (96%-100%) ethanol to each bottle.

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

D5538-00	Add 20 ml absolute (96%-100%) ethanol.
D5538-01	Add 100 ml absolute (96%-100%) ethanol.
D5538-02	Add 200 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.

A. Dry Specimens (Page 5)	For processing up to 250 mg powdered tissue.
B. Fresh/Frozen Specimens (Page 7)	For processing up to 1 g fresh (or frozen) tissue.

SP Plant DNA Maxi Protocol For 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 up to 250 mg of dried tissue into a 50 ml centrifuge tube (not supplied) and grind using a pellet pestle. 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.

1. **To up to 250 mg powdered dry tissue add 7 ml Buffer SP1 followed by** addition of 20 µl RNase A stock solution. Mix throughly by vortexing at maxi speed for 20 seconds. Make sure to disperse all clumps.

   Note: Make sure to disperse all tissue clumps by pipetting or votexing. Clumped tissues will not be lysed properly and will result in lower DNA yields.

2. **Incubate at 65°C for 30 min to 60 minutes.** Mix sample by inverting the tube several times during incubation.

3. **Add 2.5 ml Buffer SP2 and vortex to mix. Incubate the samples for 10 minutes on ice.**

4. **Centrifuge at 3000-5000 x g for 6 min at room temperature.**

   Note: Some plant materials can generate very viscous lysates and large amounts of precipitates during this step. The preparation of a cleared lysate is essential to prevent clogging of the HiBind® DNA Maxi column. For some plant sample samples, it is difficult to make preparation of cleared lysate by a single centrifugation step because not all particulate matter forms a compact pellet. **Omega Homogenizer Columns** can efficiently remove most cell debris and precipitates in next step; therefore they make the preparation of a cleared lysate rapid and efficient.

5. **Carefully aspirate the supernatant to an Omega Homogenizer Column, making sure not to disturb the pellet.**

6. **Centrifuge at 3,,000-5,000 x g for 5 min.** Longer centrifugation does not improve yields. The Omega Homogenizer Column will remove most precipitates and cell debris, but a small amount might pass through and form a pellet in the collection tube. Be careful not disturb this pellet in Step 7.

7. **Carefully transfer the cleared lysate into a new 50 ml centrifuge tube; make sure not to dislodge the pellet.** Measure the volume of the lysate for next step.

8. **Adjust binding conditions of the sample by adding 1.5 volume of Buffer SP3/ethanol mixture and immediately vortex to obtain a homogeneous mixture.** If precipitation can be seen at this point, break the precipitation by passing through a needle using a syringe or pipetting up and down 10-15 times.

9. **Transfer 15 ml of the mixture (including any precipitation) to a HiBind® DNA maxi column placed in a 50 ml collection tube (supplied) .** Centrifuge the column at 3,000-5,000 x g for 5 min to bind DNA. Discard the flow-through liquid and re-use the collection tube for Step 10.

10. **Repeat Step 9 by transfer the remainding of the mixture into the column, if any. Discard flow-through and reuse collection tube.**

11. **Place the column back into the collection tube and add 10 ml SPW Buffer.** Centrifuge at 3,000-5,000 x g for 4 min and discard the flow-through liquid. Reuse the collection tube in Step 12 below.

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

12. **Repeat wash step with an additional 10 ml SPW Buffer.** Centrifuge at 3,000-5,000 x g for 3 min. Discard flow-through; reuse collection tube in Step 13.

13. **Place the column back into 50 ml collection tube. Centrifuge empty column at 5,000 x g for 10 min to dry HiBind® Matrix.**

    *This step is **critical** for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications*

14. **Transfer the column into a new 50 ml tube (not supplied). Apply 2 ml Elution Buffer pre-warmed to 65°C directly onto HiBind® Matrix and incubate at room temperature for 5 min.** Centrifuge at 3,000-5,000 x g for 3 min to elute DNA. Smaller volumes will significantly increase DNA concentration but result in lower yields.

15. **Repeat Step 14 with an additional 2 ml of Elution buffer.** This step may be performed using another 50 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. Alternatively, DNA concentration can be increased by using the first eluate for a second elution.

SP Plant DNA Maxi Protocol For 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 1 g. Best results are obtained with young leaves or needles.

To prepare samples collect tissue in a 30 ml mortar and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using a clean pestle. 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 (up to 1 g) in a 50 ml centrifuge tube (not supplied) and immediately add 5 ml Buffer SP1 followed by addition of 20 µl RNase A stock solution.** Mix thoroughly by vortexing. Make sure to disperse all clumps.

   Note: Make sure to disperse all tissue clumps by pipetting or votexing. Clumped tissues will not be lysed properly and will result in lower DNA yields.

2. **Incubate at 65°C for 30 min. Mix sample few times by inverting tube during incubation.**

3. **Add 2 ml Buffer SP2 and vortex to mix. Incubate the samples for 10 minutes on ice.**

4. **Centrifuge at 3,000-5,000 x g for 6 min at room temperature.**

   Note: Some plant materials can generate very viscous lysates and large amounts of precipitates during this step. The preparation of a cleared lysate is essential to prevent clogging of the HiBind® DNA column. For some plant sample samples, it is difficult to make preparation of cleared lysate by a single centrifugation step because not all particulate matter forms a compact pellet. **Omega Homogenizer Columns** can efficiently remove most cell debris and precipitates in next step; therefore they make the preparation of a cleared lysate rapid and efficient.

5. **Carefully aspirate supernatant to an Omega Homogenizer Column, making sure not to disturb the pellet.**

6. **Centrifuge at 3,000-5,000 x g for 5 min.** Longer centrifugation does not improve yields. Omega Homogenizer Column will remove most precipitates and cell debris, but a small amount might pass through and form a pellet in the collection tube. Be careful not disturb this pellet in Step 7.

7. **Following Dry Samples Protocol Step 7-15 on page 5-6.**

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.	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.
	Incomplete precipitation following addition of P2.	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 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 3).
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 8 min at maximum speed.