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Introduction

The E.Z.N.A.™ Plant DNA Midi Kit allows rapid and reliable isolation of high-quality total cellular DNA from a wide variety of plant species and tissues. Up to 500 mg of wet tissue (or 150 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. 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.™ Plant DNA Midi Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. 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. Contaminants are further removed by isopropanol precipitation of DNA. Binding conditions are then adjusted and the sample is applied to an HiBind™ DNA Midi 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.™ Plant DNA Midi 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 P3. 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.

Binding Capacity

Each HiBind™ DNA Midi Column can bind up to 0.5 mg of genomic DNA. Use More than 500mg Fresh plant samples is not recommend.

Kit Contents

Product Number	D3487-00	D3487-01	D3487-02
Purification Times	2 Preps	10 Preps	25 Preps
HiBind™ DNA Midi Columns	2	10	25
15 ml Collection Tubes	2	10	25
Buffer P1	10ml	50 ml	120 ml
Buffer P2	5 ml	10 ml	25ml
Buffer P3	5 ml	20 ml	50 ml
DNA Wash Buffer Concentrate	5 ml	20 ml	2 x 20 ml
Instruction Booklet	1	1	1

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.™ Plant DNA Midi Kit procedure.

- Prepare an RNase stock solution at 20 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.

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

D3487-00	Add 20 ml absolute (96%-100%) ethanol.
D3487-01	Add 80 ml absolute (96%-100%) ethanol to each bottle.
D3487-02	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 in 60 minutes.

A. Dry Specimens	For processing ~150 mg powdered tissue.
B. Fresh/Frozen Specimens	For processing ≤1 g g fresh (or frozen) tissue.
C. Short protocol	Rapid protocol for dried or fresh samples.

E.Z.N.A. Plant DNA Midi Protocol For Dry Specimens

Materials to be provided by user:
- Centrifuge capable of at least 8,000 x g
- Nuclease-free 15 ml or 20 ml high speed centrifuge tubes (Polycarbonate thick wall centrifuge tube prefered)
- Water bath equilibrated to 65°C
- Equilibrate sterile dH_2O water or 10 mM Tris pH 9.0 at 65°C.
- Isopropyl alcohol (isopropanol)
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 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 period 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 ~100 mg of dried tissue and grind using a mortar and 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 cleaning. 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 50-150 mg powdered dry tissue add 4 ml Buffer P1.** Vortex vigorously to mix. Make sure to disperse all clumps.

2. **Incubate at 65°C for 30-60 min. Mix sample twice during incubation by inverting tube.**

3. **Add 700 µl Buffer P2 and vortex to mix. Incubate on ice for 10 minutes. Centrifuge at ≥10,000 x g for 10 min.**

4. **Carefully aspirate supernatant to a new 15ml high speed centrifuge tube making sure not to disturb the pellet or transfer any debris. Add 0.7 volume isopropanol and vortex to precipitate DNA.** This step will remove much of the polysaccharides and improves spin-column performance by increasing DNA binding capacity (and hence yield) in the steps that follow. No incubation is required after addition of isopropanol.

   TIP: In most cases 3 ml supernatant can easily be removed. This will require 2.1 ml isopropanol. Note that depending on the sample, the volume of supernatant may vary. After transferring to a fresh tube, measure the volume and add the correct amount of isopropanol**.**

5. **Immediately centrifuge at ≥ 10000 x g for 15 min at 4°C to pellet DNA.** Longer centrifugation does not improve yields.
6. **Carefully aspirate or decant the supernatant and discard making sure**

not to loose the DNA pellet. Invert the centrifuge tube on a paper towel for 5 min to allow residual liquid to drain. It is not necessary to dry the DNA pellet.

7. **Add 1.5 ml of sterile deionized water pre-heated to 65°C and vortex to resuspend the pellet.**. A brief incubation at 65°C may be necessary to effectively dissolve the DNA. Add 100 µl RNase (20 mg/ml) and mix. No additional incubation is required for RNase treatment.

   TIP: While incubating at 65°C to dissolve the DNA, label and place the required number of HiBind™ DNA Midi columns in 15 ml collection tubes.

8. **Adjust binding conditions of the sample by adding 750 µl Buffer P3 followed by 1.5 ml absolute ethanol and vortex to obtain a homogeneous mixture.** A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

9. **Apply the entire sample (including any precipitate that may have formed) to an HiBind™ DNA column placed in a 8 ml collection tube (supplied) .** Centrifuge the column at 4,000 x g for 5 min to bind DNA. Discard flow-through liquid and reuse the collection tube.

10. **Place column back into collection tube and wash by adding 3.5 ml Wash Buffer diluted with absolute (96%-100%) ethanol.** Centrifuge at 4,000 x g for 5 min and discard the flow-through liquid. Reuse the collection tube in step 11 below.

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

11. **Repeat wash step with an additional 3.5 ml Wash Buffer.** Centrifuge at 4,000 x g for 5 min. Discard flow-through and reuse 2 ml collection tube in step 12.

12. **Centrifuge empty column 15 min at 8000-10000xg 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 another clean 15 ml collection tube (Not supplied with the kit). Apply 500 µl 10 mM Tris buffer pH 9.0 (or sterile deionized water) pre-warmed to 65°C and incubate at room temperature for 3 min. Centrifuge at 8,000 x g for 10 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. It is not recommended to use larger than 2 ml of buffer for elution.

14. **Repeat step 13 with an additional 500 µl of TE buffer.** This may be performed using another 15 ml collection tube (not supplied)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 10 min before elution.

E.Z.N.A. Plant DNA Midi Protocol For Fresh/Frozen Specimens

Materials to be provided by user:

- Centrifuge capable of 8,000 x g
- Nuclease-free 10-15ml centrifuge tubes
- Water bath equilibrated to 65°C
- Equilibrate sterile dH_2O water or 10 mM Tris pH 9.0 at 65°C.
- Absolute (96%-100%) ethanol
- Isopropyl alcohol (isopropanol)
- Liquid nitrogen for freezing/disrupting samples
- RNase A stock solution at 20 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 $\leq$1 g. 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 30ml mortar and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using a clean pestles. 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 cleaning. 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 500 mg) in a 15 ml tube and mmediately add 3ml µl Buffer P1.** Vortex vigorously to mix. Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.

2. **Incubate at 65°C for 30-60 min. Mix sample twice during incubation by inverting tube.**

3. **Add 700 µl Buffer P2 and vortex to mix. Incubate on ice for 10 minutes. Centrifuge at $\geq$8,000 x g for 10 min.**

4. **Carefully aspirate cleared lysate to a new high speed centrifuge tube making sure not to disturb the pellet or transfer any debris. Add 0.7 volume isopropanol and vortex to precipitate DNA.** This step will remove much of the polysaccharides and improves spin-column performance by

increasing DNA binding capacity (and hence yield) in the steps that follow. No incubation is required after addition of isopropanol.

TIP: In most cases 2.5 ml supernatant can easily be removed. This will require 1.75ml isopropanol. Note that depending on the sample, the volume of supernatant may vary. After transferring to a fresh tube, measure the volume and add the correct amount of isopropanol.

5. **Immediately centrifuge at 8,000 x g for 15 min to pellet DNA.** Longer centrifugation does not improve yields.

6. **Carefully aspirate or decant the supernatant and discard making sure not to loose the DNA pellet.** Invert the centrifuge tube on a paper towel for 5 min to allow residual liquid to drain. It is not necessary to dry the DNA pellet.

7. **Add 1.5 ml of sterile deionized water pre-heated to 65°C and vortex to resuspend the pellet.**. A brief incubation at 65°C may be necessary to effectively dissolve the DNA. Add 50 µl RNase (20 mg/ml) and mix. No additional incubation is required for RNase treatment.

   TIP: While incubating at 65°C to dissolve the DNA, label and place the required number of HiBind™ DNA columns in 8 ml collection tubes(supplied).

8. **Adjust binding conditions of the sample by adding 750 µl Buffer P3 followed by 1.5 absolute ethanol and vortex to obtain a homogeneous mixture.** A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

9. **Apply the entire sample (including any precipitate that may have formed) to an HiBind™ DNA Midicolumn placed in a 8 ml collection tube (supplied) .** Centrifuge the column at 8,000 x g for 10 min to bind DNA. Discard the flow-through liquid and reuse the collection tube.

10. **Place column back into collection tube and wash by adding 3.50 ml Wash Buffer diluted with absolute (96%-100%) ethanol.** Centrifuge at 8,000 x g for 10 min and discard the flow-through liquid. Reuse the collection tube in step 11 below.

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

11. **Repeat wash step with an additional 3.5ml Wash Buffer.** Centrifuge at 8,000 x g for 5 min. Discard flow-through and reuse 8 ml collection tube in step 12.
12. **Centrifuge empty column 10 min at 8,000-10,000 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 15 ml tube (not supplied with this kit). Apply 500 µl 10 mM Tris buffer pH 9.0 (or sterile deionized water) pre-warmed to 65°C and incubate at room temperature for 1 min. Centrifuge at 8000-10,000 x g for 10 min to elute DNA.** Smaller volumes will significantly

increase DNA concentration but give lower yields. It is not recommended to use larger than 2ml l of buffer for elution.

14. **Repeat step 13 with an additional 0.5 ml 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, 100-50 0µg DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 1 g fresh leaf tissue.

C. Short Protocol

This simplified method allows rapid isolation of DNA from fresh, frozen, or dried specimens for use in PCR reactions. The procedure limits the amount of starting material, so that DNA yields will generally be lower than those obtained with protocols A and B.

Materials to be provided by user:

- Centrifuge capable of 8000-10,000 x g
- Nuclease-free high speed 10-15 ml centrifuge tubes
- Waterbath equilibrated to 65°C
- Sterile dH_2O water or 10 mM Tris pH 9.0 equilibrated at 65°C
- Absolute (96%-100%) ethanol
- Liquid nitrogen for freezing/disrupting fresh samples
- RNase A stock solution at 20 mg/ml

Follow suggestions for preparation of dried or fresh samples as outlined in sections A and B (pages 4 and 6 respectively). Note the following limitations on sample size:

- **Dry Samples - use a maximum of 150 mg ground tissue**
- **Fresh Samples - use a maximum of 500 mg fresh/frozen ground tissue**

1. **Collect ground sample in a centrifuge tube and add 3ml Buffer P1, 100 µl RNase A**. Vortex vigorously to mix.

2. **Incubate at 65°C for at least 20 min.** Mix sample once during incubation by inverting tube.

3. **Add 700 µl Buffer P2 and vortex to mix. Incubate on ice for 10 minutes. Centrifuge at 8000-10,000 x g for 10 min.**

4. **Carefully aspirate about 3ml supernatant to a new centrifuge tube making sure not to disturb the pellet or transfer any debris. Add ½ volume of Buffer P3 and one volume of absolute ethanol.** Vortex thoroughly to obtain a homogeneous mixture. A precipitate may form but will not affect the procedure.

   TIP: Volume of supernatant will vary, and is usually lower with dried samples. For 600 µl of supernatant add 300 µl Buffer P3 followed by 600 µl absolute ethanol.

5. **Apply 3.75 ml of the mixture to an HiBind™ DNA column assembled in a 8 ml collection tube (supplied)**. Centrifuge at 8000-10,000 x g for 5 min to bind DNA. Discard flow-through liquid and reuse collection tube in the next step.

6. **Add the remainder of the sample (including any precipitate that may have formed) to the column**. Centrifuge at 10,000 x g for 5 min and the flow-through liquid and reuse the collection tubes.

7. Place the column back into same 8 ml tube and add 3.50ml Wash Buffer diluted with absolute ethanol. Centrifuge at 8000-10, 000 x g for 5 min and discard flow-through liquid. Keep 8 ml tube.

   NOTE: Wash Buffer Concentrate must be diluted with absolute ethanol before use. Follow directions on bottle.

8. **Repeat wash step with an additional 3.5 ml Wash Buffer.** Centrifuge at 8000-10,000 x g for 5 min. Discard flow-through and reuse 8 ml collection tube in step 9.

9. **Centrifuge empty column 10 min at 8000-10,000 x g to dry.** This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.

10. **Transfer column to a clean 15 ml tube. Add 500 µl 10 mM Tris buffer pH 9.0 (or sterile deionized water) pre-warmed to 65°C and incubate at room temperature for 5 min. Centrifuge at 8000-10,000 x g for 5 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. It is not recommended to use larger than 2 ml of buffer for elution.

11. **Repeat step 10 with an additional 500 µl of buffer.** This may be performed using another 15 ml collection tube (Not supplied) 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 10 min before elution.

Yields vary according to sample size and whether dried or fresh.

Troubleshooting Guide

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 P3 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 P1 and P2 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 P1.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers P1 and P2.
	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 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.

Ordering Information

Product	Description
Plant and Fungal DNA	
D3486-01	E.Z.N.A.™ Plant DNA Miniprep Kit, 50 isolations
D3486-02	E.Z.N.A.™ Plant DNA Miniprep Kit, 200 isolations
D3487-01	E.Z.N.A.™ Plant DNA Midiprep Kit, 10 Isolations
D3487-02	E.Z.N.A.™ Plant DNA Midiprep Kit, 25 Isolations
D3488-01	E.Z.N.A.™ Plant DNA Maxiprep Kit, 5 Isolations
D3488-01	E.Z.N.A.™ Plant DNA Maxiprep Kit, 20 isolations
D3490-01	E.Z.N.A.™ Fungal DNA Miniprep Kit, 50 isolations
D3490-02	E.Z.N.A.™ Fungal DNA Miniprep Kit, 200 isolations
Plant and Fungal RNA	
R6627-01	E.Z.N.A.™ Plant RNA Kit, 50 isolations
R6627-02	E.Z.N.A.™ Plant RNA Kit, 200 isolations
R6640-01	E.Z.N.A.™ Fungal RNA Kit, 50 isolations
R6640-02	E.Z.N.A.™ Fungal RNA Kit, 200 isolations