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Introduction

The E.Z.N.A.™ High Performance (HP) Plant DNA Midi Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from fresh and dried plant tissue samples rich in polysaccharides or lower DNA contents. 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 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.™ High Performance (HP) Plant DNA Midi Kit for the first time, please read this booklet to become familiar with the procedures. This procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding of Omega Bio-Tek's HiBind® matrix. Samples are homogenized and lysed in a high salt buffer containing CTAB and extracted with chloroform to remove polysaccharides and other components that interfere with many routine DNA isolations and downstream applications. Binding conditions are then adjusted and DNA is further purified using HiBind ® DNA spin columns. In this way salts, proteins and other contaminants are removed to yield high quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization techniques.

Storage and Stability

All components of the E.Z.N.A.™ HP Plant DNA Kit are stable for at least 24 months from date of purchase when stored at 22oC-25oC. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer CPL and Buffer CXD. It is possible to dissolve such deposits by warming the solution at 37oC, though we have found that they do not interfere with overall performance.

Kit Contents

Product Number	D2487-00	D2487-01	D2487-02
HiBind® DNA Midi Columns	2	10	25
15 mL Collection Tubes	2	10	25
Buffer CPL	10 mL	40 mL	150 mL
Buffer CXD	5 mL	15 mL	40 mL
DNA Wash Buffer	12 mL	40 mL	3 x 40 mL
DNA Elution Buffer	5 mL	15 mL	60 mL
Instruction Booklet	1	1	1

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.™ High Performance Plant DNA 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**.

D2487-00	Add 18 mL absolute (96%-100%) ethanol.
D2487-01	Add 60 mL absolute (96%-100%) ethanol to each bottle.
D2487-02	Add 60 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 ~150 mg powdered tissue.
B. Fresh/Frozen Specimens (Page 6)	For processing ≤500 mg fresh (or frozen) tissue.
C. Lower DNA content samples (Page 9)	For processing up to 250 mg dried or 1gram fresh (or frozen) tissue.

A. E.Z.N.A.™ High Performance Plant DNA Dry Specimen Protocol

Materials to be provided by user

- ✓ Centrifuge capable of at least 6,000 x g
- ✓ Swinging-bucket rotor for 15 ml tubes
- ✓ Nuclease-free 15 mL centrifuge tubes
- ✓ chloroform:isoamyl alcohol (24:1)
- ✓ Waterbath equilibrated to 65oC
- ✓ Equilibrate Elution Buffer or water at 65oC.
- ✓ Isopropyl alcohol (isopropanol)
- ✓ Absolute (96%-100%) ethanol
- ✓ RNase A stock solution at 20 mg/mL
- ✓ Optional: 2-mercaptoethanol

This is the most robust method for isolation of total cellular (mitochondrial, chloroplast, and genomic) DNA.

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 ~150 mg of dried tissue into a mortar and ground the sample with 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. 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 50-100 mg powdered dry tissue add 3 ml Buffer CPL in a 15 mL microfuge tube.** Optional: Add 50 µl 2-mercaptoethanol per sample and vortex vigorously to mix. Make sure to disperse all clumps.

   TIP: Process in sets of four to six tubes: grind, add Buffer CPL and 2-mercaptoethanol, and proceed to Step 2 before starting another set. Initially, do not exceed 150 mg dried tissue. Amount can be increased according to results.

2. **Incubate at 65°C for 30 min. Mix sample twice during incubation by inverting tube.** Optional: If necessary, add 10 µL of RNase into the lysate before incubation to remove the RNA.

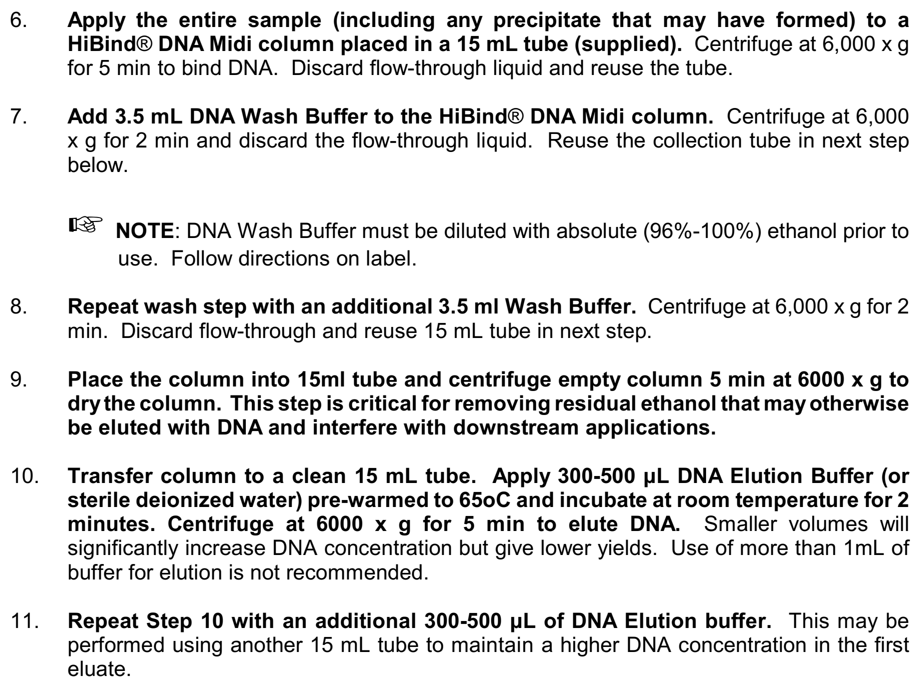
3. **Add 3 mL chloroform/Isoamyl alcohol (24:1) and vortex to mix. Centrifuge at ≥6,000 x g for 15 min.**

4. **Carefully aspirate 2 mL supernatant to a new 15 mL centrifuge tube making sure not to disturb the pellet or transfer any debris.**

5. **Adjust binding conditions of the sample by adding 1 mL Buffer CXD followed by 2mL absolute ethanol and vortex to obtain a homogeneous mixture.** A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

☞ **NOTE**: This the point to start the optional vacuum/spin protocol. (See Page 10 for

details.)



6. **Apply the entire sample (including any precipitate that may have formed) to a HiBind® DNA Midi column placed in a 15 mL tube (supplied).** Centrifuge at 6,000 x g for 5 min to bind DNA. Discard flow-through liquid and reuse the tube.

7. **Add 3.5 mL DNA Wash Buffer to the HiBind® DNA Midi column.** Centrifuge at 6,000 x g for 2 min and discard the flow-through liquid. Reuse the collection tube in next step below.

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

8. **Repeat wash step with an additional 3.5 ml Wash Buffer.** Centrifuge at 6,000 x g for 2 min. Discard flow-through and reuse 15 mL tube in next step.

9. **Place the column into 15ml tube and centrifuge empty column 5 min at 6000 x g to dry the column. 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. Apply 300-500 µL DNA Elution Buffer (or sterile deionized water) pre-warmed to 65oC and incubate at room temperature for 2 minutes. Centrifuge at 6000 x g for 5 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 1mL of buffer for elution is not recommended.

11. **Repeat Step 10 with an additional 300-500 µL of DNA Elution buffer.** This may be performed using another 15 mL tube to maintain a higher DNA concentration in the first eluate.

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

B. E.Z.N.A.™ High Performance Plant DNA Fresh/Frozen Specimen Protocol

Materials to be provided by user

- ✓ Centrifuge capable of 6,000 x g
- ✓ Swinging-bucket rotor for 15 ml tubes
- ✓ Nuclease-free 15 mL centrifuge tubes
- ✓ Water bath equilibrated to 65oC
- ✓ Equilibrate Elution Buffer or sterile dH2O at 65oC.
- ✓ Absolute (96%-100%) ethanol
- ✓ Isopropyl alcohol (isopropanol)
- ✓ chloroform:isoamyl alcohol (24:1)
- ✓ Liquid nitrogen for freezing/disrupting samples
- ✓ RNase A stock solution at 20 mg/mL
- ✓ Optional: 2-mercaptoethanol

☞ **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 gram. Best results are obtained with young leaves or needles.

To prepare samples collect tissue in a 15 mL or 50 mL microfuge tube and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using pestles. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70oC 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 immediately add 2.5 mL Buffer CPL.** Optional: Add 50 µL 2-mercaptoethanol and vortex vigorously. Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.

   TIP: Process in sets of 2 to four tubes: fill all tubes with liquid nitrogen, grind, add Buffer CPL and 2-mercaptoethanol; proceed to Step 2 before starting another set. As a starting point use 500 mg tissue per tube and if yield and purity are satisfactory increase to 1 gram

2. Incubate at 65°C for 15 min. Mix sample twice during incubation by inverting tube. Optional: If necessary, add 10 µL of RNase into the lysate before incubation to remove the RNA.

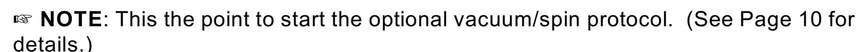
3. **Add 2.5 mL chloroform/Isoamyl alcohol (24:1) and vortex to mix. Centrifuge at 6,000 x g for 10 min.**

4. **Carefully aspirate 2mL supernatant to a new 15 mL tube making sure not to disturb the pellet or transfer any debris.**

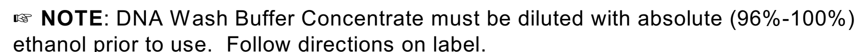
5. **Adjust binding conditions of the sample by adding 1 mL Buffer CXD followed by**

2 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.

6. **Apply the entire sample (including any precipitate that may have formed) to a HiBind® DNA Midi column placed in a 15 mL collection tube (supplied) .** Centrifuge the column at 6,000 x g for 5 min to bind DNA. Discard flow-through liquid and reuse the tube.

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

7. **Add 3.5 ml DNA wash Buffer (diluted with absolute ethanol) into the HiBind® DNA Midi column.** Centrifuge at 6,000 x g for 2 min and discard the flow-through liquid. Reuse the collection tube in next step below.

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

8. **Repeat wash step with an additional 3.5 ml Wash Buffer.** Centrifuge at 6,000 x g for 5 min. Discard flow-through and reuse 15 mL collection tube in next step below.

9. **Place the column into 15 collection tube and centrifuge empty column 5 min at 6000 x g to dry the column. This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.**

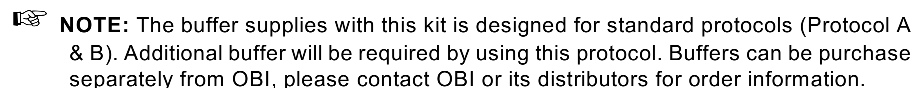
10. **Transfer column to a clean 15 mL tube. Apply 300-500 µL Elution Buffer or sterile deionized water, pre-warmed to 65oC and incubate at room temperature for 2 min. Centrifuge at 6,000 x g for 5 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 1mL of buffer for elution is not recommended.

11. **Repeat Step 10 with an additional 300-500 µL of DNA 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 60oC-70oC for 5 min before elution.

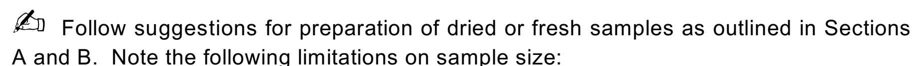
C. E.Z.N.A.™ High Performance Plant DNA Protocol for Lower DNA Content Samples

This modified method allows rapid isolation of DNA from fresh, frozen, or dried specimens for sample types with lower DNA content or when larger yields are essential. The procedure increases the amount of starting material so that DNA yields will generally be higher than those obtained with Protocols A and B.


NOTE: The buffer supplies with this kit is designed for standard protocols (Protocol A & B). Additional buffer will be required by using this protocol. Buffers can be purchase separately from OBI, please contact OBI or its distributors for order information.

Materials to be provided by user

- ✓ Swinging-bucket centrifuge capable of 6,000 x g
- ✓ Centrifuge capable of 6,000 x g
- ✓ Nuclease-free 15ml tubes
- ✓ Waterbath equilibrated to 65oC
- ✓ Elution Buffer or sterile dH2O water or equilibrated at 65oC
- ✓ chloroform:isoamyl alcohol (24:1)
- ✓ Absolute (96%-100%) ethanol
- ✓ Liquid nitrogen for freezing/disrupting fresh samples
- ✓ RNase A stock solution at 20 mg/mL
- ✓ Optional: 2-mercaptoethanol


Follow suggestions for preparation of dried or fresh samples as outlined in Sections A and B. Note the following limitations on sample size:

- ✓ **Dry Samples - use a maximum of 250 mg ground tissue**
- ✓ **Fresh Samples - use a maximum of 1 g fresh/frozen ground tissue**

1. **Collect ground sample in a 15 mL polypropylene tube and add 9.0 mL Buffer CPL. Optional:** Add 10 µL 2-mercaptoethanol per mL Buffer CPL and vortex to vigorously to mix**.** Incubate at room temperature for 60 min.

2. A**dd 9 mL chloroform/Isoamyl alcohol (24:1) and vortex to mix. Centrifuge at 6,000 x g for 10 min.**

3. **Transfer the top top aqueous phase into a new 15 mL tube.**

4. **Add 0.7 volume isopropanol and vortex to precipitate DNA.**

5. **Immediately centrifuge at 6,000 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 dislodge the DNA pellet.** Place inverted 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 65oC, to each tube and vortex to resuspend the pellet.** Add 30 µL RNase (20 mg/mL) and mix. A brief incubation at 65oC may be necessary to effectively dissolve the DNA.

8. **Adjust binding conditions of the sample by adding 0.75 mL Buffer CXD 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 entire sample from step 8 to a HiBind® DNA midi-column assembled in a 15mL collection tube (supplied)**. Centrifuge at 6,000 x g for 5 min to bind DNA. Discard flow-through liquid and reuse collection tube in the next step.

10. Place the column in a new 15 ml centrifuge tube and add 3.5 mL DNA Wash Buffer diluted with absolute ethanol. Centrifuge at 6,000 x g for 5 min and discard flow-through liquid. Re-use the 15 ml tube for next step.

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

11. **Repeat wash step with an 3.5 mL Wash Buffer.** Centrifuge at 6,000 x g for 5 min. Discard flow-through and reuse 15 mL tube.

12. **Centrifuge empty column at 6000 x g for 5 minute to dry the column.** 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. Add 300-500 µL DNA Elution Buffer (or sterile deionized water) pre-warmed to 65oC and incubate at room temperature for 1 min. Centrifuge at 6000 x for 5 minutes to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 1

mL of buffer for elution is not recommended.

14. **Repeat Step 13 with an additional 300-500 µL of DNA Elution buffer.** This may be performed using another 15 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.

D. Vacuum/Spin Protocol for Plant DNA Isolation

☞ **Note:** Please read through previous section of this book before using this protocol.

1. Prepare wet or dry samples by following the standard Protocol in previous sections until loading DNA/CDX/Ethanol mixture to HiBind ® DNA column.

2. Prepare the vacuum manifold according to manufacturer' s instruction and connect the V-Spin column to the manifold.

3. Load the DNA/CXD/Ethanol solution to the column.

4. Switch on vacuum source to draw the sample through the column and turn off the vacuum.

5. Wash the column by adding 750 µL DNA wash buffer, draw the wash buffer through the column by turn on the vacuum source. Repeat this step with another 750 µL DNA wash buffer.

6. Assemble the column into a 2 mL collection tube and transfer the column to a micro centrifuge. Spin 1 minute to dry the column.

7. Place the column in a clean 1.5 mL microcentrifuge tube and add 150 µL DNA elution Buffer or water. Centrifuge at maximum speed for 1 minute to elute DNA.

Troubleshooting

Problem	Cause	Suggestions
Clogged column	Carry-over of debris.	Following extraction with chloro:isoamyl alcohol, make sure no particulate material is transferred.
	DNA pellet not completely dissolved before applying sample to column.	In Protocols C, ensure that DNA is dissolved in water before adding Buffer CXD and ethanol. This may need repeated incubation at 65oC and vortexing.
	Sample too viscous.	Do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers CPLand CXD and use two or more columns per sample.
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer CPL
	Poor lysis of sample.	Decrease amount of starting material or increase amount of Buffers CPL, chlorosoamyl alcohol,and CXD.
	DNA remains bound to column.	Increase elution volume to 200 µL and incubate on column at 65oC 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.	DNA 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.