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Introduction

The E.Z.N.A.™ High Performance (HP) Plant DNA 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 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 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 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 Mini Kit, except the RNase A are stable for at least 24 months from the date of purchase when stored at 22°C-25°C. Store RNase A at -20°C. 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 37°C, though we have found that they do not interfere with overall performance.

Kit Contents

Product Number	D2485-00	D2485-01	D2485-02
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
Buffer CPL	5 mL	50 mL	150 mL
Buffer CXD	2 mL	10 mL	40 mL
SPW Wash Buffer	2.5 mL	25 mL	3 x 25 mL
RNase A	60 µl	550µl	2.1ml
Elution Buffer	1.5 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.

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

  D2485-00 Add 10 mL absolute (96%-100%) ethanol.

  D2485-01 Add 100 mL absolute (96%-100%) ethanol to bottle

  D2485-02 Add 100 mL absolute (96%-100%) ethanol per 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 Yields range from 10µg to more than 50µg/100mg dry tissue.

  B. Fresh/Frozen Specimens (Page 6) — For processing ≤100 mg fresh (or frozen) tissue. Yield is similar to A.

  C. Lower DNA content samples (Page 9) — For processing up to 200 mg dried or 450 mg fresh (or frozen) tissue. Yield is similar to A.

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

A. Dry Specimens

Materials to be provided by user:

- Nuclease-free 1.5 mL microfuge tubes
- chloroform:isoamyl alcohol (24:1)
- Waterbath equilibrated to 65°C
- Absolute (96%-100%) ethanol
- Optional: 2-mercaptoethanol

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 45°C oven, powdered, and stored dry at room temperature. To prepare dried samples, place ~50 mg of dried tissue into a 2.0 mL microfuge tube 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 10-50 mg powdered dry tissue add 600 µl Buffer CPL in a 1.5 mL microfuge tube.** Mix throughly by vortexing. Make sure to disperse all clumps.
   Optional: For some plant containing very high level of polyphenol, add 10 ul 2-mercaptoethanol.
   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 50 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.**

3. **Add 600 µL chloroform/Isoamyl alcohol (24:1) and vortex at maxi speed for 20 seconds. Centrifuge at ≥10,000 x g for 10 min at room temperature.**

4. **Carefully aspirate 300 µL supernatant to a new 1.5 mL microfuge tube making sure not to disturb the pellet or transfer any debris.**

5. **OPTIONAL:** Certain tissues such as leaves have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. Add 10µl RNase A and incubate at room temperature for 10-20 minutes. Proceed with the protocol.

6. **Adjust binding conditions of the sample by adding 150 µL Buffer CXD followed by 300 µL absolute ethanol and vortex to obtain a homogeneous mixture.** A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.
   Tips: Buffer CXD and ethanol can be premixed. For 1 ml Buffer CXD, add 2 ml absolute ethanol. Then add 1.5 volume of Buffer CXD/Ethanol Mixture (450ul) into the lysate. Vortex to mix well.

7. **Apply the entire sample (including any precipitate that may have formed) to a HiBind® DNA column placed in a 2.0 mL collection tube (supplied).** Centrifuge the column at 10,000 x g for 1 min to bind DNA. Discard both the 2.0 mL collection tube and the flow-through liquid.

8. **Transfer column to a second collection tube and wash by adding 650 µL SPW Wash Buffer diluted with absolute ethanol.** Centrifuge as above and discard the flow-through liquid. Reuse the collection tube in next step. **NOTE**: SPW Wash Buffer must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.

9. **Repeat wash step with an additional 650 µL SPW Wash Buffer.** Centrifuge as above. Discard flow-through and reuse collection tube in next step.

10. **Place the column into collection tube and centrifuge empty column 2 min at 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.*

11. **Transfer column to a clean 1.5 mL tube. Apply 50-100 µL Elution Buffer (pre-warmed to 65°C) and incubate for 3 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.

12. **Repeat Step 11 with an additional 50-100 µL of Elution buffer.** This may be performed using another 1.5 mL tube to maintain a higher DNA concentration in the first eluate.

B. Fresh/Frozen Specimens

Materials to be provided by user:

- Water bath equilibrated to 65°C
- Absolute (96%-100%) ethanol
- chloroform:isoamyl alcohol (24:1)
- Liquid nitrogen for freezing/disrupting samples
- 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 ≤200 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 VWR (Cat# KT749521-0500). 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 100 mg) in a 1.5 mL microfuge tube and immediately add 500 µL Buffer CPL.** Mix throughly by vortexing. Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.

   Optional: For some plant containing very high level of polyphenol, add 10 ul 2-mercaptoethanol.

   TIP: Process in sets of four to six 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 100 mg tissue per tube and if yield and purity are satisfactory increase to 200 mg.

2. **Incubate at 65°C for 15 min.** Mix sample twice during incubation by inverting tube.

3. **Add 500 µL chloroform/Isoamyl alcohol (24:1) and vortex at maxi speed for 20 seconds.** Centrifuge at ≥10,000 x g for 10 min at room temperature.

4. **Carefully aspirate 300 µL supernatant to a new 1.5 mL microfuge tube making sure not to disturb the pellet or transfer any debris.**

   OPTIONAL: Certain tissues such as leaves have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. Add 10µl RNase A and incubate at room temperature for 10-20 minutes. Proceed with the protocol.

5. **Adjust binding conditions of the sample by adding 150 µL Buffer CXD followed by 300 µL absolute ethanol and vortex to obtain a homogeneous mixture.** A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

   Tips: Buffer CXD and ethanol can be premixed. For 1 ml Buffer CXD, add 2 ml absolute ethanol. Then add 1.5 volume of Buffer CXD/Ethanol Mixture (450ul) into the lysate. Vortex to mix well.

6. **Apply the entire sample (including any precipitate that may have formed) to a HiBind® DNA column placed in a 2.0 mL collection tube (supplied) .** Centrifuge the column at 10,000 x g for 1 min to bind DNA. Discard both the collection tube and the flow-through liquid.

7. **Transfer column to a second collection tube and wash by adding 650 µL SPW Wash Buffer diluted with absolute (96%-100%) ethanol.** Centrifuge as above and discard the flow-through liquid. Reuse the collection tube in next step below.

   NOTE: SPW Wash Buffer must be diluted with absolute ethanol prior to use. Follow directions on label.

8. **Repeat wash step with an additional 650 µL SPW Wash Buffer.** Centrifuge as above. Discard flow-through and reuse collection tube in next step below.

9. **Place the column into collection tube and Centrifuge empty column 2 min at 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 1.5 mL tube. Apply 50-100 µL Elution Buffer, pre-warmed to 65°C and incubate for 3 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.

11. **Repeat Step 10 with an additional 50-100 µL Elution buffer.** This may be performed using another 1.5 mL tube to maintain a higher DNA concentration in the first eluate.

C. Protocol For Samples With Lower DNA Contents

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:

- Nuclease-free microfuge tubes
- Nuclease-free 15 mL and 50 mL tubes
- Waterbath equilibrated to 65°C
- Sterile dH_2O water or 10 mM Tris, pH 9.0 or 8.5 equilibrated at 65°C
- chloroform:isoamyl alcohol (24:1)
- Absolute (96%-100%) ethanol
- Liquid nitrogen for freezing/disrupting fresh samples
- Optional: 2-mercaptoethanol

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 200 mg ground tissue**

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

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

2. **Add 4.5 mL chloroform/Isoamyl alcohol (24:1) and vortex to mix. Centrifuge at 3,000 x g for 10 min.**

3. **Carefully aspirate top aqueous phase to a new 15mL tube making sure not to disturb the pellet or transfer any debris. Add 4.5 mL chloroform/Isoamyl alcohol (24:1) and vortex to mix. Centrifuge at 3,000 x g for 10 min.**

4. **Transfer the top top aqueous phase into a new 15 mL tube. Add 0.7 volume isopropanol and vortex to precipitate DNA.**

5. **Immediately centrifuge at 3,000 x g for 20 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 microfuge tube on a paper towel for 1 min to allow residual liquid to drain. It is not necessary to dry the DNA pellet.

7. **Add 400 µL of sterile deionized water, pre-heated to 65°C, to each tube and vortex to resuspend the pellet.** Add 20 µL RNase and mix. Incubate at 55°C for 30 minutes.
   TIP: While incubating at 55°C to dissolve the DNA, label and place the required number of HiBind ® DNA columns in 2 mL collection tubes

8. **Adjust binding conditions of the sample by adding 200 µL Buffer CXD followed by 400 µL 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 700 µL of the mixture to a HiBind® DNA column assembled in a 2 mL collection tube (supplied)**. Centrifuge at 10,000 x g for 1 min to bind DNA. Discard flow-through liquid and reuse collection tube in the next step.

10. **Add the remainder of the sample (including any precipitate that may have formed) to the column**. Centrifuge at 10,000 x g for 1 min and discard both the 2 mL collection tube and the flow-through liquid.

11. Place the column in a second 2 mL tube and add 650 µL Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min and discard flow-through liquid. Keep 2 mL tube.

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

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

13. **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.

14. **Transfer column to a clean 1.5 mL tube. Add 100 µL DNA Elution Buffer (or sterile deionized water) pre-warmed to 65°C and incubate at room temperature for 1 min. Centrifuge at Maximum speed 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.

15. **Repeat Step 14 with an additional 100 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 60°C-70°C for 5 min before elution. Yields vary according to sample size and whether dried or fresh. Between 2 µg-10 µg restrictable DNA can usually be obtained with this method.

Vacuum/Spin Protocol for Plant DNA Isolation (V-Spin column only)

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 650 µL SPW wash buffer, draw the wash buffer through the column by turn on the vacuum source. Repeat this step with another 650 µL SPW 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 50-100 µL Elution Buffer. 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 65°C 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 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.	SPW 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.