Contents

Introduction

E.Z.N.A.™ Plant Miniprep Kits allow rapid and reliable isolation of high-quality total cellular DNA from a wide variety of plant species and tissues. 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 HiBind™ matrix with the speed and versatility of spin column technologyto 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 Miniprep 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 spin-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.

New in this edition

- New introduced V-Spin column (#D3485) features an attached cap and a standard outlet luer at the bottom. The attached cap virtually assures the elimination of potential contamination.
- Optional vaccum/spin protocol is available for V-Spin column

Storage and Stability

All components of the E.Z.N.A.™ Plant Miniprep 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.

Kit Contents

Product Number	D3486-00 D3485-00	D3486-01 D3485-01	D3486-02 D3485-02
HiBind™ DNA Columns	5	50	200
2 ml Collection Tubes	10	100	400
Buffer P1	5 ml	50 ml	180 ml
Buffer P2	1ml	10 ml	40 ml
Buffer P3	2 ml	20 ml	80 ml
DNA Wash Buffer Concentrate	12 ml	48 ml	2 x 60 ml
Instruction Booklet	1	1	1

Before Starting

- ! Please read the entire booklet to become familiar with the E.Z.N.A.™ Plant Miniprep 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**.

D3486/D3485-00, Trial Kit	Add 18 ml absolute (96%-100%) ethanol.
D3486/D3485-01, 50 preps	Add 72 ml absolute (96%-100%) ethanol to each bottle.
D3486/D3485-02, 200 preps	Add 90 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 ~50 mg powdered tissue. Yield is sufficient for several tracks on Southern assay.
B. Fresh/Frozen Specimens (page 6)	For processing ≤200 mg fresh (or frozen) tissue. Yield is similar to A.
C. Short protocol (page 9)	Rapid protocol for dried or fresh samples. Yield is sufficient for PCR.

Plant Miniprep Protocol

A. Dry Specimens

Materials to be provided by user:

- ! Microcentrifuge capable of at least 10,000 x g
- ! Nuclease-free 1.5 ml or 2 ml microfuge tubes
- ! Waterbath equilibrated to 65°C
- ! Equilibrate sterile dH_2O water or 10 mM Tris pH 9.0 at 65°C.
- ! 2-mercaptoethanol
- ! 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 ~50 mg of dried tissue into a microfuge (2 ml tubes are recommended for processing of >50 mg tissue) 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 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 10-50 mg powdered dry tissue add 800 µl Buffer P1.** Add 10 µl 2-mercaptoethanol and vortex vigorously to mix. Make sure to disperse all clumps.

   TIP: Process in sets of four to six tubes: grind, add Buffer P1 and 2-mercaptoethanol, and proceed to step 2 before starting another set. Do not exceed 50 mg dried tissue.

2. **Incubate at 65°C for 10 min. Mix sample twice during incubation by inverting tube.**

3. **Add 140 µl Buffer P2 and vortex to mix. Centrifuge at 10,000 x g for 10 min.**

4. **Carefully aspirate supernatant to a new microfuge 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 700 µl supernatant can easily be removed. This will require 490 µl 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 10,000 x g for 2 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 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 300 µl 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 20 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 2 ml collection tubes.

8. **Adjust binding conditions of the sample by adding 150 µl Buffer P3 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.

9. **Apply the entire sample (including any precipitate that may have formed) to an HiBind™ DNA column placed in a 2 ml collection tube (supplied) .** Centrifuge the column at 10,000 x g for 1 min to bind DNA. Discard both the 2 ml collection tube and the flow-through liquid.

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

10. **Transfer column to a second collection tube and wash by adding 750 µl Wash Buffer diluted with absolute (96%-100%) ethanol.** Centrifuge at 10,000 x g for 1 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 750 µl Wash Buffer.** Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse 2 ml collection tube in step 12.

12. **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.

13. **Transfer column to a clean 1.5 ml tube. Apply 100 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 10,000 x g for 1 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. It is not recommended to use larger than 200 µl of buffer for elution.

14. **Repeat step 13 with an additional 100 µl 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, 10-50 µg DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 50 mg dried tissue.

B. Fresh/Frozen Specimens

Materials to be provided by user:

- Microcentrifuge capable of 10,000 x g
- Nuclease-free microfuge tubes
- Water bath equilibrated to 65°C
- Equilibrate sterile dH_2O water or 10 mM Tris pH 9.0 at 65°C.
- 2-mercaptoethanol
- 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$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 microfuge tube and immediately add 600 µl Buffer P1.** Add 10 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 four to six tubes: fill all tubes with liquid nitrogen, grind, add Buffer P1 and 2-mercaptoethanol, and 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 10 min. Mix sample twice during incubation by inverting tube.**

3. **Add 140 µl Buffer P2 and vortex to mix. Centrifuge at 10,000 x g for 10 min.**

4. **Carefully aspirate cleared lysate to a new microfuge 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 600 µl supernatant can easily be removed. This will require 420 µl 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 10,000 x g for 2 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 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 300 µl 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 20 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 2 ml collection tubes.

8. **Adjust binding conditions of the sample by adding 150 µl Buffer P3 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.

9. **Apply the entire sample (including any precipitate that may have formed) to an HiBind™ DNA column placed in a 2 ml collection tube (supplied) .** Centrifuge the column at 10,000 x g for 1 min to bind DNA. Discard both the 2 ml collection tube and the flow-through liquid.

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

10. **Transfer column to a second collection tube and wash by adding 750 µl Wash Buffer diluted with absolute (96%-100%) ethanol.** Centrifuge at 10,000 x g for 1 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 750 µl Wash Buffer.** Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse 2 ml collection tube in step 12.
12. **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.

13. **Transfer column to a clean 1.5 ml tube. Apply 100 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 10,000 x g for 1 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. It is not recommended to use larger than 200 µl of buffer for elution.

14. **Repeat step 13 with an additional 100 µl 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, 20-50 µg DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 200 mg 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. Thus in most cases there may not be sufficient material for Southern analysis or cloning work.

Materials to be provided by user:

- Microcentrifuge capable of 10,000 x g
- Nuclease-free microfuge tubes
- Waterbath equilibrated to 65°C
- Sterile dH_2O water or 10 mM Tris pH 9.0 equilibrated at 65°C
- 2-mercaptoethanol
- 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 10 mg ground tissue**
- **Fresh Samples - use a maximum of 40 mg fresh/frozen ground tissue**

1. **Collect ground sample in a microfuge tube and add 600 µl Buffer P1 and 20 µl RNase (20 mg/ml)**. Vortex vigorously to mix and incubate at room temperature for 1 min. Add 10 µl 2-mercaptoethanol and vortex to mix.

2. **Incubate at 65°C for at least 5 min.** Mix sample once during incubation by inverting tube.

3. **Add 140 µl Buffer P2 and vortex to mix. Centrifuge at 10,000 x g for 10 min.**

4. **Carefully aspirate 600 µl supernatant to a new microfuge 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 800 µl of the mixture to an HiBind™ DNA column assembled in a 2ml 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.

6. **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 2ml collection tube and the flow-through liquid.

7. Place the column in a second 2ml tube and add 750 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.

8. **Repeat wash step with an additional 750 µl Wash Buffer.** Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse 2 ml collection tube in step 9.

9. **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.

10. **Transfer column to a clean 1.5 ml tube. Add 100 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 10,000 x g for 1 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. It is not recommended to use larger than 200 µl of buffer for elution.

11. **Repeat step 10 with an additional 100 µl 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.

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)(#D3485)

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.**
2. Prepare the vacuum manifold according to manufacturer' s instruction and connect the V-Spin column to the manifold.
3. Load the DNA/P3/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 30-50µl TE or water. Stand for 1-2 minute and centrifuge 1 minute to elute DNA.

Troubleshooting

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 No.	Product Name	Description
Plant DNA and Plant RNA Isolation Kits		
D3485-01/02 D3486-01/02	Plant DNA Miniprep Kit	Isolation of total cellular DNA from dry and wet plant samples
D3487-01/02	Plant DNA Midiprep Kit	Isolation of total cellular DNA from up to 500 mg plant samples
D3488-01/02	Plant DNA Maxiprep Kit	Isolation of total cellular DNA from up to 2 gram dry and wet plant samples
R6627-01/02 R6827-01/02	Plant RNA Kit	Isolate total cellular RNA from plant samples
R6628-01/02	Plant RNA Midiprep Kit	Isolate up to 800ug total cellular RNA from 800plant samples
Fungal DNA and Fungal RNA Kit		
D3490-01/02 D3390-01/02	Fungal DNA Miniprep Kit	Isolation of total cellular DNA from dry and wet fungal samples
D3590-01/02	Fungal DNA Midiprep Kit	Isolation of total cellular DNA from up to 500 mg fungal samples
R6640-01/02 R6840-01/02	Fungal RNA Kit	Isolate total cellular RNA from fungal samples