omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Plant DNA Kit

D3485-00	5 preps
D3485-01	50 preps
D3485-02	200 preps

Manual Date: August 2020
Revision Number: v7.1

For Research Use Only

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E.Z.N.A.® Plant DNA Kit

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Introduction and Overview

The E.Z.N.A.® Plant DNA Kit allows for the rapid and reliable isolation of high-quality total cellular DNA from a wide variety of plant species and tissues. Up to 200 mg wet tissue (or 50 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of Omega Bio-tek's 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 applications. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

If using the E.Z.N.A.® Plant DNA 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 lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated. Contaminants are further removed by an isopropanol precipitation step. Binding conditions are adjusted and the sample is transferred to a HiBind® DNA Mini Column. Two rapid wash steps remove trace contaminants such as residual polysaccharides and pure DNA is eluted in water or low ionic strength buffer.

New in this Edition:

August 2020

- An optional protocol modification to improve yields has been added at the DNA pellet resuspension step.

March 2018

- An optional centrifugation step has been added to the protocols to help remove debris that may have been transferred following lysis and debris precipitation steps.

December 2017

- VAC-08, Omega's vacuum manifold had been discontinued and is no longer available for purchase. Compatible vacuum manifolds are listed on Page 9.

April 2013

- Equilibration Buffer (used in the Troubleshooting section) is no longer included with this kit. Equilibration Buffer can be replaced with 3M NaOH provided by the user.

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

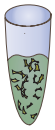
DNA concentration = Absorbance 260 × 50 × (Dilution Factor) µg/mL

A value greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations.

If necessary the DNA can be concentrated. Add sodium chloride to reach a final concentration of 0.1M followed by 2 volumes of 100% ethanol. Mix well and incubate at -20°C for 10 minutes. Centrifuge at 10,000 x *g* for 15 minutes and aspirate and discard the supernatant. Add 700 µL 70% ethanol and centrifuge at 10,000 x *g* for 2 minutes. Aspirate and discard the supernatant, air dry the pellet for 2 minutes, and resuspend the DNA in 20 µL sterile deionized water or 10 mM Tris-HCl, pH 8.5.

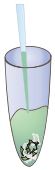
Illustrated Protocol

Grind Sample in Liquid Nitrogen

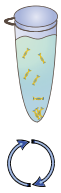


Lyse

Precipitate Polysaccharides



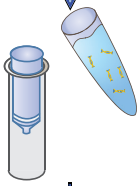
Transfer Cleared Supertanant



Precipitate DNA

Resuspend DNA

Adjust Binding Conditions



Transfer to a HiBind® DNA Mini Column

Wash 2X

Dry

Elute

Kit Contents

Product Number	D3485-00	D3485-01	D3485-02
Purifications	5 Preps	50 Preps	200 Preps
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
P1 Buffer	5 mL	50 mL	180 mL
P2 Buffer	1 mL	10 mL	40 mL
P3 Buffer	4 mL	20 mL	80 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL
Elution Buffer	2 mL	30 mL	60 mL
RNase A	40 µL	250 µL	1 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Plant DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Store RNase A at 2-8°C. Store all other components at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve any deposits by warming the solution at 37°C with gently shaking.

- Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3485-00	10 mL
D3485-01	100 mL
D3485-02	100 mL per bottle

Protocol Selection

Different protocols are listed depending on the state of plant specimen (dried or fresh/frozen). Choose the most appropriate protocol to follow. In addition, a short protocol is given for isolation of DNA for PCR reactions.

Protocol	Description
Dried Specimens (Page 10)	For processing ≤50 mg of dried tissue, yield is sufficient for several tracks on Southern assay.
Fresh/Frozen Specimens (Page 14)	For processing ≤200 mg of fresh or frozen tissue, yield is sufficient for several tracks on Southern assay.
Short Protocol (Page 18)	Rapid protocol for dried or fresh samples. Yield is sufficient for PCR.

Disruption of Plant Tissues

1. Grind samples with pestle
   A) Dried 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 ~15 mg dried tissues into a microcentrifuge tube (1.5 mL tubes are recommended) and grind using a pellet pestle. Disposable Kontes pestles work well and are available from SSIbio (Cat# 1004-39 & 1005-39). 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. A fine powder will ensure optimal DNA extraction and yield.

   B) Fresh/Frozen Specimens
   Due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to ~50 mg for first time users. It is very important to not overload the HiBind® DNA Mini Column. Too much starting material will decrease the yield and purity due to inefficient lysis. However, for some plant species, increasing the starting material can increase DNA yield. We recommend starting with 50 mg tissue. If results obtained are satisfactory, then increase amount of starting material. **Best results are obtained with young leaves or needles.**

   Although various means of sample disruption can be used for this kit, such as beads or pestles, we recommend grinding the sample in liquid nitrogen. To prepare samples, collect tissue in a 1.5 mL or 2 mL microcentrifuge tube and dip the tube in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable Kontes pellet pestles. Disposable Kontes pellet pestles are available from SSIbio (Cat# 1005-39). Alternatively, allow the liquid nitrogen to evaporate and store the 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. Transfer the ground sample into a 1.5 mL microcentrifuge tube.

   Note: Do not allow the sample to thaw during handling and weighing. To prevent the sample from thawing, keep the samples on dry ice.

2. Disrupt Samples with Commercial Homogenizers

   Fresh, frozen, and dried plant tissue can be effectively disrupted and homogenized by rapid agitation in the presence of beads.

   For Fresh, Frozen, and Lyophilized/Dried Tissue

   1. Add one 3-4 mm stainless steel bead to each well of a 96-well round-well plate, or add two 3-4 mm stainless steel bead to a 2.0 mL homogenizer tube.

   2. Close the individual tubes with cap strips of the plate, or screw the cap onto the homogenizer tube.

   3. Optional: Freeze the sample in liquid nitrogen.

      Note: Lyophilized/dried samples do not require freezing with liquid nitrogen.

   4. Place the racks or plates into the clamps of the homogenizer.

   5. Homogenize for 60-90 seconds at 30 Hz. Tissue samples are disrupted and simultaneously homogenized with the shearing and crushing action of the beads. Refer to manufacturer's protocol regarding use of liquid nitrogen with the homogenizer.

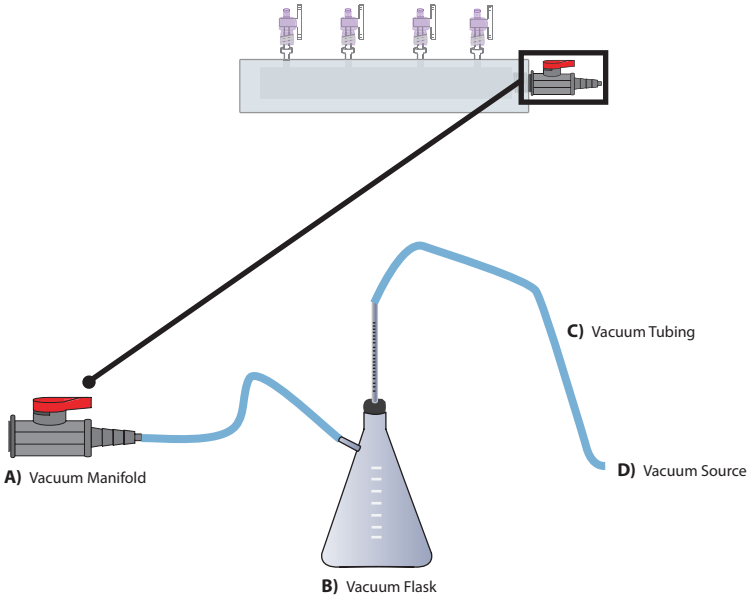
Vacuum Settings

The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold
Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Conversion from millibars:	Multiply by:
millimeters of mercury (mmHg)	0.75
kilopascals (kPa)	0.1
inches of mercury (inHg)	0.0295
Torrs (Torr)	0.75
atmospheres (atm)	0.000987
pounds per square inch (psi)	0.0145

Illustrated Vacuum Setup:



E.Z.N.A.® Plant DNA Kit - Dried Plant Samples Protocol

This is the most robust method for the isolation of total cellular (mitochondrial, chloroplast, and genomic) DNA. Yields are usually sufficient for several tracks on a Southern blot for RFLP mapping.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000*g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 65°C
- Vortexer
- Sterile deionized water
- 100% isopropanol
- 100% ethanol
- Homogenization equipment
  - Pestles (Recommended SSIbio, Cat# 1004-39 or 1005-39)
  - Commercial homogenizer
- Paper towels

Before Starting:

- Prepare DNA Wash Buffer according to Preparing Reagents Section on Page 6.
- Set a water bath, incubator, or heat block to 65°C.
- Heat sterile deionized water to 65°C.
- Heat Elution buffer to 65°C.

1. Prepare dried tissue samples. Refer to Pages 7-8 for details.

2. Transfer 10-50 mg dried powdered tissue to a nuclease-free 1.5 mL microcentrifuge tube (not provided).

   Note: Process in sets of four to six tubes: grind, add P1 Buffer and proceed to Step 4 before starting another set.

3. Add 800 µL P1 Buffer. Vortex to mix thoroughly. Make sure to disperse all clumps.

   Note: Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.

4. Incubate at 65°C for 10 minutes. Mix samples twice during incubation by inverting the tube.

5. Add 140 µL P2 Buffer. Vortex to mix thoroughly.

6. Centrifuge at 10,000*g* for 10 minutes.

7. Transfer cleared lysate to a new 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet. Measure the volume of the lysate.

Optional: Centrifuge at 10,000*g* for 1-2 minutes to collect any debris that may have been transferred in Step 7. Transfer the cleared lysate to a new 1.5 mL microcentrifuge tube before proceeding to Step 8.

8. Add 0.7 volumes 100% isopropanol. Vortex to precipitate DNA. This step removes most of the polysaccharides and improves mini column performance by increasing DNA binding capacity (and hence yield) in the steps that follow. No incubation is required after addition of isopropanol.

   Note: In most cases 700 µL supernatant can easily be removed. This will require 490 µL isopropanol. Depending on the sample, the volume of supernatant may vary. After transferring to a new tube, measure the volume and add the correct amount of isopropanol.

9. Immediately centrifuge at 14,000*g* for 2 minutes to pellet DNA. Longer centrifugation time does not improve yield.

10. Carefully aspirate or decant and discard the supernatant. Make sure not to dislodge the DNA pellet.

11. Invert the microcentrifuge tube on a paper towel for 1 minute to allow any residual liquid to drain. It is not necessary to dry the DNA pellet.

12. Add 300 µL sterile deionized water heated to 65°C. Vortex to resuspend the pellet. A brief incubation at 65°C may be necessary to completely dissolve the DNA.

    Note: For better DNA yield recovery, incubate samples at 65°C for 30 minutes. Vortex samples every 5 minutes for 30 minutes to completely dissolve the DNA.

13. Add 4 µL RNase A. Vortex to mix thoroughly. No additional incubation is required for RNase treatment.

    Note: RNase A can be added to the sterile deionized water (in proper proportion) in Step 12 to simplify the procedure. RNase A will remain stable during incubation.

14. Add 150 µL P3 Buffer and 300 µL 100% ethanol. Vortex immediately to obtain a homogenous mixture. A precipitate may form at this point; it will not interfere with DNA isolation. Passing the mixture through a syringe needle 3-4 times or by pipetting up and down 10-15 times may break up the precipitates.

Optional: This is the starting point of the optional Vacuum Protocol. If use of a vacuum is desired, please refer to the Vacuum Protocol found on Page 21. If not using a vacuum manifold, proceed to Step 15.

15. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

16. Transfer the entire sample, including any precipitates that may have formed, to the HiBind® DNA Mini Column.

17. Centrifuge at 10,000*g* for 1 minute.

18. Discard the filtrate and the 2 mL Collection Tube.

19. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

20. Add 650 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

21. Centrifuge at 10,000*g* for 1 minute.

22. Discard filtrate and reuse the collection tube.

23. Repeat Steps 20-22 for a second DNA Wash Buffer wash step.

24. Centrifuge the empty HiBind® DNA Mini Column at 10,000*g* for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

25. Transfer the HiBind® DNA Mini Column into a new 1.5 mL microcentrifuge tube.

26. Add 50-100 µL Elution Buffer heated to 65°C.

27. Let sit at room temperature for 3-5 minutes.

28. Centrifuge at 10,000*g* for 1 minute.

29. Repeat Steps 26-28 for a second elution step.

    Note: Each elution step will typically yield of 60-70% of the DNA bound to the column. Thus two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. In some instances yields may be increased by incubating the column at 65°C (rather than at room temperature) upon addition of Elution Buffer.

30. Store eluted DNA at -20°C.

E.Z.N.A.® Plant DNA Kit - Fresh/Frozen Plant Samples Protocol

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. This method isolates sufficient DNA for several tracks on a standard Southern assay.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000*g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 65°C
- Vortexer
- Sterile deionized water
- 100% isopropanol
- 100% ethanol
- Homogenization equipment
    - Pestles (Recommended SSIbio, Cat# 1004-39 or 1005-39)
    - Commercial homogenizer
    - Liquid nitrogen
- Paper towels

Before Starting:

- Prepare DNA Wash Buffer according to Preparing Reagents Section on Page 6.
- Set a water bath, incubator, or heat block to 65°C.
- Heat sterile deionized water to 65°C.
- Heat Elution buffer to 65°C.

1. Grind 100 mg fresh/frozen tissue samples in liquid nitrogen. Refer to Pages 7-8 for details.

   Note: Process in sets of four to six tubes: grind, add P1 Buffer and proceed to Step 4 before starting another set.

2. Transfer ground tissue to a nuclease-free 1.5 mL microcentrifuge tube (not provided).

3. Add 600 µL P1 Buffer. Vortex to mix thoroughly. Make sure to disperse all clumps.

   Note: Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.

4. Incubate at 65°C for 10 minutes. Mix samples twice during incubation by inverting the tube.

5. Add 140 µL P2 Buffer. Vortex to mix thoroughly.

6. Centrifuge at 10,000*g* for 10 minutes.

7. Transfer cleared lysate to a new 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet. Measure the volume of the lysate.

Optional: Centrifuge at 10,000*g* for 1-2 minutes to collect any debris that may have been transferred in Step 7. Transfer the cleared lysate to a new 1.5 mL microcentrifuge tube before proceeding to Step 8.

8. Add 0.7 volumes 100% isopropanol. Vortex to precipitate DNA. This step removes most 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.

   Note: In most cases 600 µL supernatant can easily be removed. This will require 420 µL isopropanol. Depending on the sample, the volume of supernatant may vary. After transferring to a new tube, measure the volume and add the correct amount of isopropanol.

9. Immediately centrifuge at 14,000*g* for 2 minutes to pellet DNA. Longer centrifugation time does not improve yield.

10. Carefully aspirate or decant and discard the supernatant. Make sure not to dislodge the DNA pellet.

11. Invert the centrifuge tube on a paper towel for 1 minute to allow any residual liquid to drain. It is not necessary to dry the DNA pellet.

12. Add 300 µL sterile deionized water heated to 65°C. Vortex to resuspend the pellet. A brief incubation at 65°C may be necessary to completely dissolve the DNA.

    Note: For better DNA yield recovery, incubate samples at 65°C for 30 minutes. Vortex samples every 5 minutes for 30 minutes to completely dissolve the DNA.

13. Add 4 µL RNase A. Vortex to mix thoroughly. No additional incubation is required for RNase treatment.

    Note: RNase A can be added to the sterile deionized water (in proper proportion) in Step 12 to simplify the procedure. RNase A will remain stable during incubation.

14. Add 150 µL P3 Buffer and 300 µL 100% ethanol. Vortex immediately to obtain a homogenous mixture. A precipitate may form at this point; it will not interfere with DNA isolation. Passing the mixture through a syringe needle 3-4 times or by pipetting up and down 10-15 times may break up the precipitates.

Optional: This is the starting point of the optional Vacuum Protocol. If use of a vacuum is desired, please refer to the Vacuum Protocol found on Page 21. If not using a vacuum manifold, proceed to Step 15.

15. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

16. Transfer the entire sample, including any precipitates that may have formed, to the HiBind® DNA Mini Column.

17. Centrifuge at 10,000*g* for 1 minute.

18. Discard the filtrate and the 2 mL Collection Tube.

19. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

20. Add 650 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

21. Centrifuge at 10,000*g* for 1 minute.

22. Discard filtrate and reuse the collection tube.

23. Repeat Steps 20-22 for a second DNA Wash Buffer wash step.

24. Centrifuge the empty HiBind® DNA Mini Column at 10,000 x *g* for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

25. Transfer the HiBind® DNA Mini Column into a new 1.5 mL microcentrifuge tube.

26. Add 50-100 µL Elution Buffer heated to 65°C.

27. Let sit at room temperature for 3-5 minutes.

28. Centrifuge at 10,000*g* for 1 minute.

29. Repeat Steps 26-28 for a second elution step.

    Note: Each elution step will typically yield of 60-70% of the DNA bound to the column. Thus two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. In some instances yields may be increased by incubating the column at 65°C (rather than at room temperature) upon addition of Elution Buffer.

30. Store eluted DNA at -20°C.

E.Z.N.A.® Plant DNA Kit - 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 therefore DNA yields generally will be lower than those obtained with the protocols for "Dried Plant Samples" (Page 10) or "Fresh/Frozen Plant Samples" (Page 14). In most cases, there may not be sufficient material for Southern analysis or cloning work.

Follow the suggestions for preparation of dried or fresh samples as outlined in the "Disruption of Plant Tissues" section on Pages 7-8.

Note the following limitations on sample size:

Dried Samples - use a maximum of 10 mg ground tissue
Fresh Samples - use a maximum of 40 mg fresh/frozen ground tissue

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 10,000*g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 65°C
- Vortexer
- 100% ethanol
- Homogenization equipment
  - Pestles (Recommended SSIbio, Cat# 1004-39 or 1005-39)
  - Commercial homogenizer
  - Liquid nitrogen

Before Starting:

- Prepare DNA Wash Buffer according to Preparing Reagents Section on Page 6.
- Set a water bath, incubator, or heat block to 65°C.
- Heat Elution buffer to 65°C.

1. Prepare tissue samples. Refer to Pages 7-8 for details.

2. Transfer ground tissue to a nuclease-free 1.5 mL microcentrifuge tube (not provided).

   Note: Process in sets of four to six tubes: grind, add P1 Buffer and proceed to Step 4 before starting another set.

3. Add 600 µL P1 Buffer. Vortex to mix thoroughly. Make sure to disperse all clumps.

   Note: Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.

4. Incubate at 65°C for at least 5 minutes. Mix samples once during incubation by inverting the tube.

5. Add 140 µL P2 Buffer. Vortex to mix thoroughly.

6. Centrifuge at 10,000*g* for 10 minutes.

7. Carefully transfer 600 µL cleared lysate to a new 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet.

   Note: If lysate is not cleared, perform a second centrifugation at 10,000 x *g* for 10 minutes to clear the lysate.

8. Add ½ volume P3 Buffer and one volume 100% ethanol. Vortex immediately to obtain a homogenous mixture. A precipitate may form at this point; it will not interfere with DNA isolation.

   Note: The volume of lysate will vary and is usually lower with dried samples. For example, for 600 µL supernatant, add 300 µL P3 Buffer and 600 µL 100% ethanol.

9. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

10. Transfer 750 µL sample, including any precipitates that may have formed, to the HiBind® DNA Mini Column.

11. Centrifuge at 10,000*g* for 1 minute.

12. Discard the filtrate and reuse the 2 mL Collection Tube.

13. Repeat Steps 10-12 until all of the sample has been transferred to the HiBind® DNA Mini Column.

14. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

15. Add 650 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

16. Centrifuge at 10,000*g* for 1 minute.

17. Discard filtrate and reuse the collection tube.

18. Repeat Steps 15-17 for a second DNA Wash Buffer wash step.

19. Centrifuge the empty HiBind® DNA Mini Column at 10,000*g* for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

20. Transfer the HiBind® DNA Mini Column into a new 1.5 mL microcentrifuge tube.

21. Add 50 µL Elution Buffer heated to 65°C.

22. Let sit at room temperature for 3-5 minutes.

23. Centrifuge at 10,000*g* for 1 minute.

24. Repeat Steps 21-23 with 100 µL Elution Buffer heated to 65°C.

    Note: This may be performed using another 1.5 mL microcentrifuge tube to maintain a higher DNA concentration in the first eluate.

25. Store eluted DNA at -20°C.

E.Z.N.A.® Plant DNA Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Vacuum manifold
- Vacuum source
- Microcentrifuge capable of at least 10,000*g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 65°C
- Vortexer
- Sterile deionized water
- 100% isopropanol
- 100% ethanol
- Homogenization equipment
  - Pestles (Recommended SSIbio, Cat# 1004-39 or 1005-39)
  - Commercial homogenizer
  - Liquid nitrogen
- Paper towels

Before Starting:

- Prepare DNA Wash Buffer according to Preparing Reagents Section on Page 6.
- Set a water bath, incubator, or heat block to 65°C.
- Heat sterile deionized water to 65°C.
- Heat Elution buffer to 65°C.

Note: Please read through previous sections of this manual before using this protocol.

1. Prepare dried or fresh/frozen samples by following Steps 1-14 of the "Dried Plant Samples" protocol (Pages 10-12) or Steps 1-14 of the "Fresh/Frozen Plant Samples" protocol (Pages 14-16).

2. Prepare the vacuum manifold according to manufacturer's instructions and connect the HiBind® DNA Mini Column to the manifold.

3. Transfer the prepared sample to the column.

4. Turn on the vacuum source to draw the sample through the column.

5. Turn off the vacuum.

6. Add 650 µL DNA Wash Buffer.

   Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 6 for instructions.

7. Turn on the vacuum source to draw the DNA Wash Buffer through the column.

8. Turn off the vacuum.

9. Repeat Steps 6-8 for a second DNA Wash Buffer wash step.

10. Transfer the HiBind® DNA Mini Column to a 2 mL Collection Tube.

11. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

12. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

13. Add 50-100 µL Elution Buffer heated to 65°C.

14. Let sit at room temperature for 3-5 minutes.

15. Centrifuge at 10,000*g* for 1 minute.

16. Repeat Steps 13-15 for a second elution step.

    Note: Each elution step will typically yield of 60-70% of the DNA bound to the column. Thus two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. In some instances yields may be increased by incubating the column at 65°C (rather than at room temperature) upon the addition of Elution Buffer.

17. Store eluted DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **800-832-8896**.

Problem	Cause	Solution
Clogged Column	Debris carryover	Following precipitation with P2 Buffer, make sure no particulate material is transferred. Perform an additional centrifugation step to clear supernatant if necessary.
	DNA pellet not completely dissolved before applying sample to column	In Dried and Fresh/Frozen Protocols, ensure that DNA is dissolved in water before adding P3 Buffer and ethanol. May need to increase incubation time at 65°C and vortex often.
	Sample too viscous	In the Short Protocol, do not exceed suggested amount of starting material. Alternatively, increase amounts of P1 Buffer and P2 Buffer and use two or more columns per sample.
	Incomplete precipitation after addition of P2 Buffer	Increase RCF or time of centrifugation after addition of P2 Buffer.
Problem	Cause	Solution
Low A_{260}/A_{280} Ratio	Salt carryover	DNA Wash Buffer must be at room temperature.
	Ethanol carryover	Following the second wash step, ensure that the column is dried. Centrifuge for 2 minutes at maximum speed.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical suppport staff, toll free, at **800-832-8896**.

Problem	Cause	Solution
Low DNA Yield	Incomplete homogenization	Completely homogenize sample.
	Poor elution	Repeat elution with increased elution volume. Incubate columns at 65°C for 5 minutes with Elution Buffer.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol before use. DNA Wash Buffer must be at room temperature.
	Sample has low DNA content	Increase starting material and volume of all reagents proportionally. Load aliquots of lysate through the column successively.
		Incubate the DNA pellet at 65°C for 30 minutes. Vortex the pellet every 5 minutes for 30 minutes to completely dissolve the DNA.
	Column matrix lost binding capacity during storage	Add 100 µL 3M NaOH to the column prior to loading sample. Centrifuge at 10,000*g* for 30 seconds. Add 100 µL water to the column and centrifuge at 10,000*g* for 30 seconds. Discard the filtrate.

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For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

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