omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Plant & Fungal DNA Kit

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

Manual Date: January 2024
Revision Number: v1.0

For Research Use Only

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E.Z.N.A.® Plant & Fungal DNA Kit

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Intended Use

For professional research use.

The E.Z.N.A.® Plant & Fungal DNA Kit is intended for isolation and purification of genomic DNA from up to 200 mg of wet sample or 50 mg dry sample.

Intended User

The E.Z.N.A.® Plant & Fungal DNA Kit is intended for professional use and to be used by or under the supervision of professional users, such as laboratory personnel, technicians, researchers and physicians specifically instructed and trained in molecular biology techniques.

Product Description

The E.Z.N.A.® Plant & Fungal DNA Kit allows for the rapid and reliable isolation of high-quality total cellular DNA from a wide variety of plant or fungal 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 and fungal 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 & Fungal DNA Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. Dry or fresh plant or fungal 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:

January 2024:

- In the newest version of this kit, the E.Z.N.A.® Fungal DNA Mini Kit (D3390) and E.Z.N.A.® Plant DNA kit (D3485) have merged.
- FG1 Buffer, FG2 Buffer, and FG3 Buffer have been discontinued and replaced with P1 Buffer, P2 Buffer, and P3 Buffer respectively. This is a name change only; the formulation has not changed.

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:

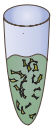
DNA concentration = A260 × 50 × (Dilution Factor) µg/mL

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 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*g* for 15 minutes and aspirate and discard the supernatant. Add 700 µL 70% ethanol and centrifuge at 10,000*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.

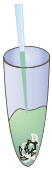
Illustrated Protocol

Homogenize or Grind Sample in Liquid Nitrogen



Lyse

Precipitate Polysaccharides

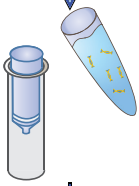


Transfer Cleared Supertanant

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	50	200
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 & Fungal DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. RNase A should be stored at 2-8°C. All remaining components should be stored at room temperature and away from bright light. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and 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 tissue 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 14)	For processing ≤50 mg of dried tissue, yield is sufficient for several tracks on Southern assay.
Fresh/Frozen Specimens (Page 18)	For processing ≤200 mg of fresh or frozen tissue, yield is sufficient to that for dried specimens.
Short Protocol (Page 22)	Rapid protocol for dried or fresh samples. Yield is sufficient for PCR.

Disruption of Plant or Fungal 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 plant tissues or ~50 mg dried fungal 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 and fungi, sample size should be limited to ~50 mg plant tissues or ≤200 mg fungal tissues 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 or fungi 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 or fungal 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.

Warnings

This kit is for research use only.

Please read all instructions carefully before using the kit.

Decontaminate and dispose of all potentially infectious materials in accordance with applicable local, state, and national regulations. Please refer to safety data sheets (SDSs) for information on disposal of different components included in this kit.

Safety Information

All chemicals and biological materials are potentially hazardous. Biological samples such as plasma, serum, tissues, body fluids, blood etc. are potentially infectious and must be treated as biohazardous materials. Conduct all work in properly equipped facilities following universal precautions and using appropriate personal safety equipment such as disposable gloves, lab coats, safety glasses etc. as required by policies and procedures outlined by your facility. Please refer to safety data sheets (SDSs) for information on safe handling, transport and disposal of different components included in this kit. SDSs are made available in PDF format on the product page at www.omegabiotek.com. Discard all waste in accordance with the local safety regulations.

Some of the buffers included in the product contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions to guanidine-containing waste.** Please access the SDSs online for detailed information on the reagents.

Precautions

Some of the buffers included in the E.Z.N.A.® Plant & Fungal DNA Kit contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions** to guanidine containing sample-preparation waste. Please access the SDSs online for detailed information on the reagents.

Component	Description
RNase A 	Contains: Ribonuclease A. Danger! Causes an allergic skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection in case of inadequate ventilation. Contaminated work clothing must not be allowed out of the workplace. INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a poison center/doctor/physician/first aider if experience respiratory symptoms. ON SKIN: Wash with plenty of water. Get medical attention if skin irritation or rash occurs. Take off contaminated clothing and wash it before reuse.
P1 Buffer 	Contains: Anionic detergent. Warning! Causes serious eye irritation. May cause an allergic skin irritation. Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing must not be allowed out of the workplace. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention if eye irritation persists. ON SKIN: Wash with plenty of water. Get medical attention if skin irritation or rash occurs. Take off contaminated clothing and wash it before reuse.
P2 Buffer 	Contains: Acetic acid glacial. Danger! Causes severe skin burns and eye damage. Do not breathe mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Wash contaminated clothing before reuse. ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. SWALLOWED: Rinse mouth. Do NOT induce vomiting. Immediately call a poison center or doctor/physician. INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Precautions

Component	Description
P3 Buffer 	Contains: Guanidine hydrochloride. Warning! Causes serious eye irritation. Harmful if swallowed. Causes skin irritation. Do not eat, drink or smoke when using this product. Wear protective gloves/protective clothing/eye protection/face protection. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention if eye irritation persists. Take off contaminated clothing and wash before resuse. ON SKIN: Wash with plenty of water and soap. Get medical advice/attention if skin irritation occurs. SWALLOWED: Rinse mouth. Call a poison center or doctor/ physician if you feel unwell.

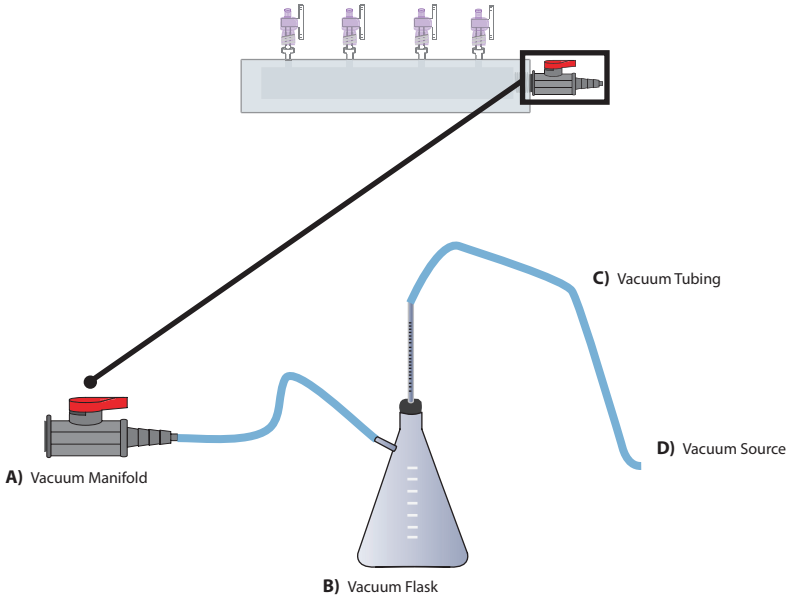
Vacuum Settings

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:



Dried Samples Protocol

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

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) OR Commercial homogenizer
- Paper towels

Before Starting:

- Prepare DNA Wash Buffer according to Preparing Reagents Section on Page 7.
- Set a water bath, incubator, or heat block to 65°C.
- Heat sterile deionized water to 65°C.
- Heat Elution buffer to 65°C.
- Prepare an ice bucket.

1. Prepare dried tissue samples. Refer to Pages 8-9 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 180 µL P2 Buffer. Vortex to mix thoroughly.

6. **For fungal samples only:** Let sit on ice for 5 minutes.

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

8. Transfer cleared lysate to a new 1.5 mL microcentrifuge tube. Do not disturb or transfer any debris.

Optional: Centrifuge at 10,000*g* for 1-2 minutes to collect any debris that may have been transferred in Step 8. Transfer the cleared lysate to a new 1.5 mL microcentrifuge tube before proceeding to Step 9.

9. Add 0.7 volumes 100% isopropanol. Vortex to precipitate DNA.

   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. This step will remove much of the polysaccharide content 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.

10. Immediately centrifuge at 14,000*g* for 2 minutes. Longer centrifugation time does not improve yield.

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

12. 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.

13. Add 300 µL sterile deionized water heated to 65°C. Vortex to resuspend the pellet.

    Note: A brief incubation at 65°C may be necessary to completely dissolve the DNA. 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.

14. 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 13 to simplify the procedure. RNase A will remain stable during incubation.

15. 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 26. If not using a vacuum manifold, proceed to Step 16.

16. Insert a HiBind® DNA Mini Column into a 2 mL collection tube.

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

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

19. Discard the filtrate and the 2 mL collection tube.

20. Transfer the HiBind® DNA Mini Column to a new 2 mL collection tube.

21. Add 750 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 7 for instructions.

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

23. Discard filtrate and reuse the collection tube.

24. Repeat Steps 21-23 for a second DNA Wash Buffer step.

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

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

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

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

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

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

30. Repeat Steps 27-29 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%. Any combination of the following steps can be used to help increase DNA yield.
    - After adding the Elution Buffer, incubate the column for 5 minutes at 65°C.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

31. Store eluted DNA at -20°C.

Fresh/Frozen 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 and fungi, sample size should be limited to ≤200 mg. Best results are obtained with young leaves or needles.

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) OR Commercial homogenizer
  - Liquid nitrogen
- Paper towels

Before Starting:

- Prepare DNA Wash Buffer according to Preparing Reagents Section on Page 7.
- Set a water bath, incubator, or heat block to 65°C.
- Heat sterile deionized water to 65°C.
- Heat Elution buffer to 65°C.
- Prepare an ice bucket.

1. Grind 100 mg fresh/frozen tissue samples in liquid nitrogen. Refer to Pages 8-9 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. **For fungal samples only:** Let sit on ice for 5 minutes.

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

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

Optional: Centrifuge at 10,000*g* for 1-2 minutes to collect any debris that may have been transferred in Step 8. Transfer the cleared lysate to a new 1.5 mL microcentrifuge tube before proceeding to Step 9.

9. Add 0.7 volumes 100% isopropanol. Vortex to precipitate DNA.

   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. This step will remove much of the polysaccharide content 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.

10. Immediately centrifuge at 14,000*g* for 2 minutes. Longer centrifugation time does not improve yield.

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

12. 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.

13. 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.

14. 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 13 to simplify the procedure. RNase A will remain stable during incubation.

15. 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 26. If not using a vacuum manifold, proceed to Step 16.

16. Insert a HiBind® DNA Mini Column into a 2 mL collection tube.

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

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

19. Discard the filtrate and the 2 mL collection tube.

20. Transfer the HiBind® DNA Mini Column to a new 2 mL collection tube.

21. Add 750 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 7 for instructions.

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

23. Discard filtrate and reuse the collection tube.

24. Repeat Steps 21-23 for a second DNA Wash Buffer step.

25. 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**.**

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

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

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

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

30. Repeat Steps 27-29 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%. Any combination of the following steps can be used to help increase DNA yield.
    - After adding the Elution Buffer, incubate the column for 5 minutes at 65°C.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

31. Store eluted DNA at -20°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 therefore DNA yields generally will be lower than those obtained with the protocols for "Dried Samples" (Page 14) or "Fresh/Frozen Samples" (Page 18). 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 Tissues" section on Pages 8-9.

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
- β-mercaptoethanol
- Homogenization equipment
  - Pestles (Recommended SSIbio, Cat# 1004-39 or 1005-39) OR commercial homogenizer
  - Liquid nitrogen

Before Starting:

- Prepare DNA Wash Buffer according to Preparing Reagents Section on Page 7.
- Set a water bath, incubator, or heat block to 65°C.
- Heat Elution buffer to 65°C.
- Prepare an ice bucket.

1. Prepare tissue samples. Refer to Pages 8-9 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 and 4 µL RNase A. 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. Let sit for 1 minute.

5. **For fungal samples only:** Add 10 µL β-mercaptoethanol. Vortex to mix thoroughly.

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

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

8. **For fungal samples only:** Let sit on ice for 5 minutes.

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

10. 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*g* for 10 minutes to clear the lysate.

11. Add 0.5 volumes P3 Buffer and 1 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.

12. Insert a HiBind® DNA Mini Column into a 2 mL collection tube.

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

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

15. Discard the filtrate and reuse the 2 mL collection tube.

16. Repeat Steps 13-15 until all of the sample has been transferred to the HiBind® DNA Mini Column.

17. Transfer the HiBind® DNA Mini Column to a new 2 mL collection tube.

18. Add 750 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 7 for instructions.

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

20. Discard filtrate and reuse the collection tube.

21. Repeat Steps 18-20 for a second DNA Wash Buffer step.

22. 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**.**

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

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

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

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

27. Repeat Steps 24-26 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.

28. Store eluted DNA at -20°C.

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 7.
- Set a water bath, incubator, or heat block to 65°C.
- Heat sterile deionized water to 65°C.
- Heat Elution buffer to 65°C.
- Prepare an ice bucket.

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

1. Prepare dried or fresh/frozen samples by following Steps 1-15 of the "Dried Samples" protocol (Pages 14-16) or Steps 1-15 of the "Fresh/Frozen Samples" protocol (Pages 18-20).

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 HiBind® DNA Mini Column.

4. Apply the vacuum until all the sample has passed through the membrane.

5. Turn off the vacuum.

6. Add 750 µL DNA Wash Buffer.

   Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 7 for instructions.

7. Apply the vacuum until all the buffer has passed through the membrane.

8. Turn off the vacuum.

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

Problem	Cause	Solution
Low DNA Yield	Incomplete disruption of starting material	Obtain a fine homogeneous powder before adding P1 Buffer.
	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.
	Polyphenol-rich samples or difficult sample types	Prior to starting the extraction, add freshly prepared PVP to a final concentration of 2% (w/v) and DTT to a final concentration of 40mM to the P1 buffer.

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling:

Picture	Description
	No additional hazards or not classified as hazardous according to GHS
 MM/DD/YYYY	Use-by date
	Check components for storage conditions.
	Lot number
	Manufacturer
	Website
	Telephone
	Fax
	Email
	LinkedIn
	Twitter
	Facebook

Notes:

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

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