omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® HP Plant DNA Mini Kit

D2485-00	5 preps
D2485-01	50 preps
D2485-02	200 preps

Manual Date: July 2019
Revision Number: v5.0

For Research Use Only

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E.Z.N.A.® HP Plant DNA Mini Kit

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Introduction and Overview

The E.Z.N.A.® High Performance (HP) DNA Mini Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from fresh, frozen, or dried plant tissue samples rich in polysaccharides or having a lower DNA content. Up to 100 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 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 applications.

If using the E.Z.N.A.® High Performance (HP) Plant DNA Mini 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, to isolate high-quality DNA. 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 adjusted and DNA is purified using a HiBind® DNA Mini Columns. 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 applications.

New in this Edition:

- CPL Buffer is now called CSPL Buffer. This is a name change only. The component has not changed.

Kit Contents

Product	D2485-00	D2485-01	D2485-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
CSPL Buffer	5 mL	40 mL	150 mL
CXD Buffer	1 mL	10 mL	40 mL
DNA Wash Buffer	2 mL	20 mL	2 x 40 mL
Elution Buffer*	5 mL	15 mL	60 mL
User Manual	✓	✓	✓

* 10 mM Tris HCl pH 8.5

Storage and Stability

All of the E.Z.N.A.® HP Plant DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in CSPL Buffer and CXD Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

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

Kit	100% Ethanol to be Added
D2485-00	8 mL
D2485-01	80 mL
D2485-02	160 mL per bottle

2. Prepare a mixture of chloroform: isoamyl alcohol (24:1)

Optional: Prepare RNase A solution at 20 mg/mL and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use. Each sample will require 2 µL RNase A.

Protocol Selection Guide

Protocol	Page	Ideal Sample
Dried Specimens	5	For processing ≤50 mg powdered tissue. Total DNA yield will 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.
Fresh or Frozen Specimens	9	For processing ≤200 mg fresh (or frozen) tissue. Yield is similar to that for dried specimens.
Specimens with Lower DNA Content	13	For processing up to 200 mg dried or 450 mg fresh (or frozen) tissue. Yields will vary according to sample size and whether dried or fresh. Between 2-10 µg DNA can usually be obtained with this method.

E.Z.N.A.® HP Plant DNA Mini Kit Protocol - Dried Specimens

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 microcentrifuge tube (2 mL tubes are recommended for processing of >50 mg tissue) and grind using a pellet pestle. Disposable Kontes pestles work well and are available from Omega Bio-tek (Cat# 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.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 12,000 x *g*
- Waterbath capable of 65°C
- Vortexer
- Nuclease-free 1.5 mL and 2.0 mL microcentrifuge tubes
- Chloroform
- Isoamyl alcohol
- 100% ethanol
- Optional: RNase A at 20 mg/mL
- Optional: 2-mercaptoethanol
- Optional: sterile deionized water

Before Starting:

- Prepare the DNA Wash Buffer according to the instructions on Page 4
- Prepare a mixture of chloroform: isoamyl alcohol (24:1)
- Heat the Elution Buffer or sterile deionized water to 65°C

1. Prepare 10-50 mg powdered dry tissue in a 1.5 or 2 mL microcentrifuge tube (not provided).

2. Add 600 µL CSPL Buffer. Vortex vigorously to mix. Make sure to disperse all clumps.

   Note: Process in sets of four to six tubes: grind, add CSPL Buffer, then proceed to Step 3 before starting another set. Do not exceed 50 mg dried tissue.

Optional: Add 10 µl 2-mercaptoethanol. Vortex vigorously to mix.

Optional: Add 2 µL RNase to the lysate before incubation to remove the RNA.

3. Incubate at 65°C for 30 minutes. Invert the samples twice during incubation.

4. Add 600 µL chloroform/isoamyl alcohol (24:1). Vortex vigorously to mix.

5. Centrifuge at ≥10,000 x *g* for 10 minutes.

6. Transfer 300 µL aqueous phase (top) to a new microcentrifuge tube, making sure not to disturb the pellet or transfer any debris.

7. Add 150 µL CXD Buffer and 300 µL 100% ethanol. Vortex to obtain a homogeneous mixture.

   Note: A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

Optional: This the point to start the optional vacuum/spin protocol. See Page 17 for details.

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

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes.
3. Centrifuge at maximum speed for 60 seconds.
4. Discard the filtrate and reuse the Collection Tube.

9. Transfer the entire sample (including any precipitate that may have formed) to the HiBind® DNA Mini Column.

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

11. Discard the filtrate and the Collection Tube.

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

13. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please refer to Page 4 or the bottle label for instructions.

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

15. Discard the filtrate and reuse the Collection Tube.

16. Repeat Steps 13-15 for a second DNA Wash Buffer wash step.

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

    Note: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.

18. Transfer the HiBind® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).

19. Add 50–100 µL Elution Buffer (or sterile deionized water) heated to 65°C.

    Note: Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

20. Centrifuge at 10,000 x *g* for 1 minute.

21. Repeat Steps 19-20 for a second elution step.

    Note: 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.
    - 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).

22. Store DNA at -20°C.

E.Z.N.A.® HP Plant DNA Mini Kit Protocol - Fresh or Frozen Specimens

This protocol is suitable for most fresh or frozen tissue samples. However, due to the tremendous variation in water and polysaccharide content of various fungi, sample size should be limited to ≤200 mg. The method isolates sufficient DNA for several tracks on a standard Southern assay.

To prepare samples, collect tissue in a 1.5 or 2 mL microcentrifuge 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 Omega Bio-tek (Cat# SSI-1015-39). 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 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.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 12,000 x *g*
- Waterbath capable of 65°C
- Vortexer
- Nuclease-free 1.5 mL and 2.0 mL microcentrifuge tubes
- Chloroform
- Isoamyl alcohol
- 100% ethanol
- Liquid nitrogen for freezing/disrupting samples
- Optional: RNase A at 20 mg/mL
- Optional: 2-mercaptoethanol
- Optional: sterile deionized water

Before Starting:

- Prepare the DNA Wash Buffer according to the instructions on Page 4
- Prepare a mixture of chloroform: isoamyl alcohol (24:1)
- Heat the sterile deionized water and Elution Buffer to 65°C

1. Prepare 100 mg tissue in a 1.5 or 2 mL microcentrifuge tube (not provided).

2. Add 500 µL CSPL Buffer. Vortex vigorously to mix. Make sure to disperse all clumps.
   Note: Process in sets of four to six tubes: grind, add CSPL Buffer, then proceed to Step 3 before starting another set. Do not exceed 200 mg fresh or frozen tissue.

Optional: Add 10 µl 2-mercaptoethanol. Vortex vigorously to mix.

Optional: Add 2 µL RNase to the lysate before incubation to remove the RNA.

3. Incubate at 65°C for 15 minutes. Invert the samples twice during incubation.

4. Add 800 µL chloroform/isoamyl alcohol (24:1). Vortex vigorously to mix.

5. Centrifuge at ≥10,000 x *g* for 5 minutes.

6. Transfer 300 µL aqueous phase (top) to a new microcentrifuge tube, making sure not to disturb the pellet or transfer any debris.

7. Add 150 µL CXD Buffer and 300 µL 100% ethanol. Vortex to obtain a homogeneous mixture.

   Note: A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

Optional: This the point to start the optional vacuum/spin protocol. See Page 17 for details.

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

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes.
3. Centrifuge at maximum speed for 60 seconds.
4. Discard the filtrate and reuse the Collection Tube.

9. Transfer the entire sample (including any precipitate that may have formed) to the HiBind® DNA Mini Column.

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

11. Discard the filtrate and the Collection Tube.

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

13. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please refer to Page 4 or the bottle label for instructions.

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

15. Discard the filtrate and reuse the Collection Tube.

16. Repeat Steps 13-15 for a second DNA Wash Buffer wash step.

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

    Note: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.

18. Transfer the HiBind® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).

19. Add 50–100 µL Elution Buffer (or sterile deionized water) heated to 65°C.

    Note: Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

20. Centrifuge at 10,000 x *g* for 1 minute.

21. Repeat Steps 19-20 for a second elution step.

    Note: 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.
    - 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).

22. Store DNA at -20°C.

E.Z.N.A.® HP Plant DNA Mini Kit Protocol - Specimens with Lower DNA Content

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 standard protocols above.

NOTE: The buffers supplied with this kit are designed for the standard protocols. Additional buffer amounts will be required for this protocol and can be purchased separately. Please contact Omega Bio-tek or its distributors for order information.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 12,000 x *g*
- Swing bucket centrifuge capable of at least 3,000 x *g*
- Waterbath capable of 65°C
- Vortexer
- Nuclease-free 15 mL and 50 mL centrifuge tubes
- Nuclease-free 1.5 mL and 2.0 mL microcentrifuge tubes
- Chloroform
- Isoamyl alcohol
- 100% isopropanol
- 100% ethanol
- Liquid nitrogen for freezing/disrupting samples
- Sterile deionized water or 10 mM Tris, pH 9.0 or 8.5
- RNase A at 20 mg/mL
- Optional: 2-mercaptoethanol

Before Starting:

- Prepare the DNA Wash Buffer according to the instructions on Page 4
- Prepare a mixture of chloroform: isoamyl alcohol (24:1)
- Heat the sterile deionized water and Elution Buffer to 65°C

Note: Follow the suggestions for preparation of dried or fresh/frozen specimens as outlined in the protocols above (Pages 5 and 9, respectively). Note the following limitations on sample size:

- Dry Samples - use a maximum of 200 mg ground tissue
- Fresh/Frozen Samples - use a maximum of 400 mg ground tissue

1. Prepare 100 mg tissue in a 15 mL centrifuge tube (not provided).

2. Add 9 mL CSPL Buffer. Vortex vigorously to mix. Make sure to disperse all clumps.
   Note: Process in sets of four to six tubes: grind, add CSPL Buffer, then proceed to Step 3 before starting another set.

Optional: Add 10 µl 2-mercaptoethanol. Vortex vigorously to mix.

3. Let sit at room temperature for 60 minutes. Invert the samples twice during incubation.

4. Add 4.5 mL chloroform:isoamyl alcohol (24:1). Vortex vigorously to mix.

5. Centrifuge at 3,000 x *g* for 10 minutes.

6. Carefully aspirate the top aqueous phase to a new 15 mL microcentrifuge tube making sure not to disturb the organic phase or transfer any debris.

7. Transfer the aqueous phase (top) to a new 15 mL centrifuge tube.

8. Add 0.7 volumes 100% isopropanol. Vortex to mix thoroughly.

9. Immediately centrifuge at 3,000 x *g* for 20 minutes. Longer centrifugation does not improve yield.

10. Carefully aspirate and discard the supernatant making sure not to dislodge the DNA pellet.

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

12. Add 400 µL sterile deionized water heated to 65°C. Vortex to resuspend the pellet.

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

13. Add 20 µL RNase A (20 mg/mL). Vortex to mix thoroughly.

14. Add 200 µL CXD Buffer and 400 µL 100% ethanol. Vortex to obtain a homogeneous mixture.

    Note: A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

Optional: This is the point to start the optional vacuum protocol. See Page 17 for details.

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

Optional Protocol for Column Equilibration

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes at room temperature.
3. Centrifuge at maximum speed for 20 seconds.
4. Discard the filtrate and reuse the Collection Tube.

16. Transfer 700 µL sample from Step 13 to the HiBind® DNA Mini Column.

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

18. Discard the filtrate and reuse the collection tube.

19. Repeat Steps 16-18 until all of the remaining sample (including any precipitates that may have formed) has been transferred to the HiBind® DNA Mini Column.

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

21. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please refer to Page 4 or the bottle label for instructions.

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

23. Discard the filtrate and reuse the Collection Tube.

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

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

    Note: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.

26. Transfer the HiBind® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).

27. Add 100 µL Elution Buffer (or sterile deionized water) heated to 65°C.

    Note: Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

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

29. Repeat Steps 27-28 for a second elution step.

    Note: 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.
    - 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).

30. Store DNA at -20°C.

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

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

Materials and Equipment to be Supplied by User:

- Vacuum Manifold

Before Starting:

- Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 4
- Heat Elution Buffer to 65°C

1. Complete Steps 1-7 of either the Dried or Fresh/Frozen Specimen Protocols or Steps 1-14 of the Protocol for Samples with Lower DNA Content (Pages 5, 9, and 13, respectively).

2. Prepare the vacuum manifold according to manufacturer's instructions.

3. Connect the HiBind® DNA Mini Column to the vacuum manifold.

Optional Protocol for Column Equilibration

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes at room temperature.
3. Turn on the vacuum to draw the NaOH through the column.
4. Turn off the vacuum.

4. Transfer the cleared supernatant from Step 7 of the Dried or Fresh/Frozen Protocols (Pages 7 and 10, respectively) or from Step 14 of the Lower DNA Content Protocol (Page 15) by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

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

6. Turn off the vacuum.

7. Add 700 µL DNA Wash Buffer.

   Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

8. Turn on the vacuum source to draw the buffer through the column.

9. Turn off the vacuum.

10. Repeat Steps 7-9 for a second DNA Wash Buffer wash step.

11. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

12. Centrifuge the empty column at maximum speed for 2 minutes.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

13. Transfer the HiBind® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).

14. Add 100 µL Elution Buffer (or sterile deionized water) heated to 65°C.

    Note: Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

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

16. Repeat Steps 14-15 for a second elution step.

    Note: 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.
    - 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).

17. Store 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 **1-800-832-8896**.

Problem	Cause	Solution
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 Protocol C, ensure that DNA is dissolved in water before adding CXD Buffer and ethanol. This may need repeated incubation at 65oC and vortexing.
	Sample too viscous.	Do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers CPLand CXD and use two or more columns per sample.
Problem	Cause	Solution
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding CSPL Buffer
	Poor lysis of sample.	Decrease amount of starting material or increase amount of CSPL Buffer, chlorosoamyl alcohol, and CXD Buffer.
	DNA remains bound to column.	Increase elution volume to 200 µL and incubate on column at 65oC for 5 min before centrifugation.
	DNA washed off.	Dilute DNA Wash Buffer by adding appropriate volume of absolute ethanol prior to use (page 3).
Problem	Cause	Solution
Problems in downstream applications	Salt carry-over.	DNA Wash Buffer must be at room temperature.
	Ethanol carry-over	Following the second wash spin, ensure that the column is dried by centrifuging 2 min at maximum speed.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (40 mL)	PDR044
RNase A (400 µL)	AC117

Notes:

Notes:

Notes:

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

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

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Plasmid


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FFPE


Fecal Matter

omega BIO-TEK

innovations in nucleic acid isolation

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