omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® HP Plant & Fungal DNA Kit

D2485-00	5 preps
D2485-02	200 preps

Manual Date: January 2024
Revision Number: v1.0

For Research Use Only

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E.Z.N.A.® HP Plant & Fungal DNA Kit

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Intended Use

For professional research use.

The E.Z.N.A.® HP Plant & Fungal DNA Kit is intended for isolation and purification of genomic DNA from up to 100 mg of wet sample or 30 mg dry sample.

Intended User

The E.Z.N.A.® HP 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.® High Performance (HP) Plant & Fungal DNA Kit is designed for efficient recovery of genomic DNA from fresh, frozen, and dried plant or fungal tissue samples rich in polysaccharides or having 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 or fungal tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques.

If using the E.Z.N.A.® HP Plant & Fungal DNA Kit for the first time, please read this booklet to become familiar with the procedures. This procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding of Omega Bio-tek's HiBind® matrix, to purify 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 further purified using HiBind ® DNA Mini Columns. In this way, salts, proteins, and other contaminants are removed to yield high-quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization.

New in this Edition:

January 2024:

- In the newest version of this kit, the E.Z.N.A.® HP Fungal DNA Mini Kit (D3195) and E.Z.N.A.® HP Plant DNA kit (D2485) have merged.

Kit Contents

Product	D2485-00	D2485-02
Purifications	5	200
HiBind® DNA Mini Columns	5	200
2 mL Collection Tubes	10	400
CSPL Buffer	5 mL	150 mL
CXD Buffer	1 mL	40 mL
DNA Wash Buffer	2.5 mL	3 x 25 mL
Elution Buffer	2 mL	60 mL
User Manual	✓	✓

Storage and Stability

All of the E.Z.N.A.® HP Plant & Fungal DNA Kit components are guaranteed for at least 12 months from the date of purchase when 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.

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	10 mL
D2485-02	100 mL per bottle

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

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

Protocol Selection

Different protocols are listed depending on the state of tissue specimen (dried or fresh/ frozen). Choose the most appropriate protocol to follow.

Protocol	Description
Dried Specimens (Page 9)	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 A260/A280 ratio of 1.7-1.9 can be isolated using 50 mg dried tissue.
Fresh/Frozen Specimens (Page 12)	For processing ≤200 mg of fresh (or frozen) tissue. Yield is similar to that for dried specimens.
Specimens with Lower DNA Content (Page 15)	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.

Processing Plant and Fungal Samples

Dry 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 ~50 mg dried tissue into a 2 mL microcentrifuge tube and grind using a pellet pestle. Disposable Kontes pestles work well. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and wiping the surface clean between samples. A fine powder will ensure optimal DNA extraction and yield.

Fresh/Frozen Specimens

To prepare fresh or frozen samples, collect tissue in a 1.5 mL 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. 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.

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.® HP 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
CSPL Buffer 	Contains: Cationic detergent. Warning! Causes serious eye irritation. 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.
CXD 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.

Dried Samples

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.

Materials and Equipment to be Supplied by User:

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

Before Starting:

- Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 5
- Prepare a mixture of chloroform: isoamyl alcohol (24:1)
- Heat sterile deionized water or Elution Buffer to 65°C

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

2. Add 600 µL CSPL Buffer. Vortex to mix thoroughly. 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. Initially, do not exceed 50 mg dried tissue. Amount can be increased according to results.

Optional: Add 10 µL 2-mercaptoethanol. Vortex to mix thoroughly.

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

3. Incubate at 65°C for 30 minutes. Invert the tube twice during incubation to mix the sample.

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

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

6. Carefully aspirate 300 µL aqueous phase (top) to a new 1.5 mL microcentrifuge tube making sure not to disturb the organic phase 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 is the point to start the optional vacuum protocol. See Page 19 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 at room temperature.
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*g* for 1 minute.

11. Discard the filtrate and the collection tube.

12. Insert the HiBind® DNA Mini Column into 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 see Page 5 for instructions.

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

15. Discard the filtrate and reuse the collection tube.

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

17. 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.

18. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

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

20. Centrifuge at maximum speed 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.

Fresh/Frozen Samples

This protocol is suitable for most fresh/frozen tissue samples allowing more efficient recovery of DNA. Due to the tremendous variation in water and polysaccharide content of various plants and fungi, sample size should be limited to ≤200 mg. The 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 12,000*g*
- Waterbath capable of 65°C
- Vortexer
- Nuclease-free 1.5 mL and 2 mL microcentrifuge tubes
- Chloroform
- Isoamyl alcohol
- 100% ethanol
- Liquid nitrogen for freezing/disrupting samples
- Optional: RNase A stock solution at 20 mg/mL
- Optional: 2-mercaptoethanol
- Optional: sterile deionized water

Before Starting:

- Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 5
- Prepare a mixture of chloroform: isoamyl alcohol (24:1)
- Heat sterile deionized water or Elution Buffer to 65°C

1. Transfer 100 mg ground fresh/frozen tissue in a 1.5 or 2 mL microcentrifuge tube (not provided).

2. Immediately add 500 µL CSPL Buffer. Vortex to mix thoroughly. 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. Initially, do not exceed 100 mg fresh/frozen tissue. Amount can be increased according to results.

Optional: Add 10 µL 2-mercaptoethanol. Vortex to mix thoroughly.

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

3. Incubate at 65°C for 15 minutes. Invert the tube twice during incubation to mix the sample.

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

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

6. Carefully aspirate 300 µL aqueous phase (top) to a new 1.5 mL microcentrifuge tube making sure not to disturb the organic phase 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 is the point to start the optional vacuum protocol. See Page 19 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 at room temperature.
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*g* for 1 minute.

11. Discard the filtrate and the collection tube.

12. Transfer the HiBind® DNA Mini Column into 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 see Page 5 for instructions.

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

15. Discard the filtrate and reuse the collection tube.

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

17. 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.

18. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

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

20. Centrifuge at maximum speed 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.

Samples 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 the previous protocols. Yields vary according to sample size and whether dried or fresh. Between 2-10 µg restrictable DNA can usually be obtained with this method.

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*g*
- Centrifuge with a swing bucket rotor capable of 3,000*g*
- Waterbath capable of 65°C
- Vortexer
- Nuclease-free 1.5 mL and 2 mL microcentrifuge tubes
- Nuclease-free 15 mL and 50 mL centrifuge tubes
- Chloroform
- Isoamyl alcohol
- 100% isopropanol
- 100% ethanol
- Sterile deionized water
- Optional: RNase A stock solution at 20 mg/mL
- Optional: 2-mercaptoethanol

Before Starting:

- Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 5
- Prepare a mixture of chloroform: isoamyl alcohol (24:1)
- Heat sterile deionized water or Elution Buffer to 65°C

Note: Follow suggestions for preparation of dried or fresh samples as outlined in the protocols above (Pages 9 and 12, 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. Transfer ground sample to a 15 mL centrifuge tube (not provided).

2. Add 9 mL CSPL Buffer. Vortex to mix thoroughly.

   Note: Process in sets of four to six tubes: grind, add CSPL Buffer, then proceed to Step 3 before starting another set. Initially, do not exceed 50 mg dried tissue. Amount can be increased according to results.

Optional: Add 10 µL 2-mercaptoethanol per 1 mL CSPL Buffer. Vortex to mix thoroughly. Make sure to disperse all clumps.

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 to mix thoroughly.

5. Centrifuge at 3,000*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. Add 0.7 volumes 100% isopropanol. Vortex to mix thoroughly.

8. Immediately centrifuge at 3,000*g* for 20 minutes.

   Note: Longer centrifugation does not improve yield.

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

10. 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.

11. 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.

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

13. 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 19 for details.

14. 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.

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

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

17. Discard the filtrate and reuse the collection tube.

18. Repeat Steps 15-17 until all of the remaining sample (including any precipitates that may have formed) has been transferred to the HiBind® DNA Mini Column.

19. Transfer the HiBind® DNA Mini Column into a new 2 mL collection tube.

20. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

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

22. Discard the filtrate and reuse the collection tube.

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

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

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

25. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL or 2 mL microcentrifuge tube (not provided).

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

    Note: Smaller volumes will significantly increase DNA concentration but result in lower yields. Using more than 200 µL for elution is not recommended.

27. Centrifuge at maximum speed for 1 minute.

28. Repeat Steps 26-27 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).

29. Store DNA at -20°C.

Vacuum Protocol

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

Materials and Equipment to be Supplied by User:

- Vacuum Manifold
- Centrifuge with a swing bucket rotor capable of 3,000*g*
- Waterbath capable of 65°C
- 100% ethanol
- Optional: sterile deionized water

Before Starting:

- Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 5
- Heat sterile deionized water or Elution Buffer to 65°C

1. Complete Steps 1-7 of either the Dried or Fresh/Frozen Tissue Protocols (Pages 9 and 12, 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 10 and 13, respectively) 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 5 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 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 clean 1.5 mL or 2 mL microcentrifuge tube (not provided).

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

    Note: Smaller volumes will significantly increase DNA concentration but result in lower yields. Using more than 200 µL for elution is not recommended.

15. Centrifuge at maximum speed 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	Debris carryover	Following extraction with chloro:isoamyl alcohol, make sure no particulate material is transferred.
	DNA pellet not completely dissolved before applying sample to column	In the Protocol for Samples with Lower DNA Content, ensure that DNA is dissolved in water before adding CXD Buffer and ethanol. This may need repeated incubations at 65°C and vortexing.
	Sample too viscous	Do not exceed suggested amount of starting material. Alternatively, increase the amounts of CSPL and CXD Buffers 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 the amount of starting material or increase the amount of CSPL Buffer, chloro: isoamyl alcohol, and CXD Buffer.
	DNA remains bound to column	Increase the elution volume to 200 µL and incubate the column at 65°C for 5 minutes before centrifugation.
	DNA washed off	Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 5.

Problem	Cause	Solution
Problems in downstream applications	Salt carryover	DNA Wash Buffer must be at room temperature.
	Ethanol carryover	Following the second wash step, centrifuge the column for 2 minutes at maximum speed to completely dry the matrix.
	RNA contamination	For Dried Specimens and Fresh/Frozen Specimens Protocol, perform the optional RNase A step at double the RNase A volume (Step 2, page 9 or page 12, respectively) and double the incubation time (Step 3, page 10 or page 13, respectively). For Specimens with lower DNA content, double the RNase A volume and incubate at 70°C for 5 minutes at Step 12, page 17.

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

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


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FFPE


Fecal Matter

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