E.Z.N.A.® Forensic DNA Kit

Table of Contents

Manual Revision: January 2016

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The E.Z.N.A.® Forensic DNA Kit is designed to provide a rapid and easy method for the isolation of genomic DNA from forensic samples such as dry blood, buccal swabs, and semen samples for consistent PCR and Southern analysis. This kit can also be used for the preparation of genomic DNA from mouse tail snips, whole blood, buffy coat, serum, and plasma. This kit can process single or multiple samples simultaneously. There is no need for phenol/chloroform extractions and time-consuming steps such as precipitation with isopropanol or ethanol are eliminated. DNA purified using the E.Z.N.A.® Forensic DNA Kit is ready for applications such as PCR*, Southern blotting, and restriction enzyme digestion.

Overview

E.Z.N.A.® Forensic DNA Kits combine the reversible DNA binding properties of the HiBind® matrix, a new silica-based material, with the speed of mini-column centrifugation to quickly purify high-quality DNA. A specifically formulated buffer system allows genomic DNA up to 50 kb to bind to the matrix. Samples are lysed under denaturing conditions and then applied to the HiBind® DNA Mini Columns. The HiBind® matrix selectively binds DNA; therefore, cellular debris, hemoglobin, and other proteins can be easily eliminated during the washing procedures. High-quality DNA is eluted in sterile deionized water or elution buffer. Each HiBind® DNA Mini Column can bind approximately 100 µg DNA from up to 30 mg tissue or $1x10^7$ cells; although, using more starting material is not recommended.

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. DNA concentration is calculated as:

[DNA] = (Absorbance260) x (0.05 µg/ µL) x (Dilution factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity. Yields vary with both amount and type of tissue used. Generally, 30 mg of fresh tissue will yield 10-40 µg DNA with two elutions of 200 µL each.

New in this Edition:

- STL Buffer has been renamed TL Buffer. This is a name change only. The buffer formulation has not changed.
- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D3591-00	D3591-01	D3591-02
Purifications	5 preps	50 preps	200 preps
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	15	150	600
BL Buffer	1.5 mL	13 mL	50 mL
TL Buffer	1.1 mL	11 mL	44 mL
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	2 mL	20 mL	3 x 25 mL
Elution Buffer	5 mL	15 mL	50 mL
OB Protease Solution	150 µL	1.4 mL	4 x 1.4 mL
User Manual	✓	✓	✓

Note: The E.Z.N.A.® Forensic DNA Kit is supplied with enough buffer for the Standard Protocol (Page 6) for dried blood, body fluids, and semen samples. Supplemental protocols that are included for specialized sample types (such as the buccal swabs, hair, mails, and saliva), may require higher buffer volumes. For these protocols, the total number of purifications that can be performed will need to be reduced. If you are using one of these protocols, additional buffers can be purchased separately from Omega Bio-tek, Inc. Please see the Ordering Information section on Page 32 of this manual, the Accessories section in our catalog, or call customer service toll-free for assistance at 1.800.832.8896.

Storage and Stability

All E.Z.N.A.® Forensic DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows: OB Protease Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store at 2-8°C. Store all other components at room temperature (22-25°C). Check buffers for precipitates before use. Redissolve any precipitates by warming to 37˚C.

Preparing Reagents

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

Kit	100% Ethanol to be Added
D3591-00	8 mL
D3591-01	80 mL per bottle
D3591-02	100 mL per bottle

2. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D3591-00	1.6 mL
D3591-01	10 mL
D3591-02	32 mL

Guidelines for Vacuum Manifold

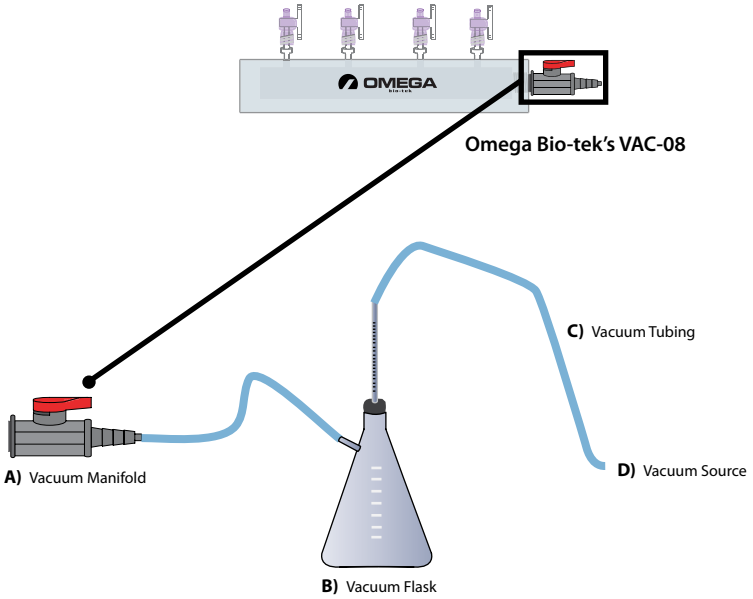
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other 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)

Manifold	Recommended Pressure (mbar)
VAC-08	-200 to -600

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.® Forensic DNA Kit Protocol - Standard Protocol

Dried blood, body fluids, and semen samples on filter paper can be processed using the following method.

Materials and Reagents to be Supplied by User:

- 100% Ethanol
- Isopropanol
- 1.5 mL or 2.0 mL nuclease-free microcentrifuge tubes
- Nuclease-free pipette tips
- Microcentrifuge capable of 14,000 x *g*
- Vortexer
- Water baths or heat blocks capable of 55°C, 60°C and 70°C
- Optional: 3M NaOH

Before Starting:

- Heat the water baths or heat blocks to 55°C and 60°C
- Heat the Elution Buffer to 70°C
- Prepare the DNA Wash Buffer and HBC Buffer according to the instructions in the Preparing Reagents section on Page 4

1. Cut or punch out the blood spot (or other sample) from the filter paper. (Up to 200 µL of blood can be used for each spot.) Tear or cut filter into small pieces and place into a microcentrifuge tube.

   Note: Use 3-4 punched circles (3 mm diameter) for each DNA isolation.

2. Add 200 µL TL Buffer. Vortex to mix thoroughly.

3. Incubate at 55°C for 15 minutes. Vortex every 2 minutes to mix.

4. Add 25 µL OB Protease Solution. Vortex to mix thoroughly.

5. Incubate at 60°C for 45 minutes with occasional mixing.

6. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

7. Add 225 µL BL Buffer. Vortex to mix thoroughly.

8. Incubate at 60°C for 10 minutes.

9. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

10. Add 300 µL isopropanol. Vortex to mix thoroughly.

11. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

12. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube provided with this kit.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit at room temperature 4 minutes.
3. Centrifuge at maximum speed for 30 seconds.
4. Discard the filtrate and reuse the collection tube.

13. Transfer the entire sample from **Step 11** to the column, including any precipitate that may have formed.

14. Centrifuge at maximum speed for 1 minute.

15. Discard the filtrate and the collection tube.

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

17. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

18. Centrifuge at maximum speed for 1 minute.

19. Discard the filtrate and the collection tube.

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 ethanol before use. Please see Page 4 for instructions.

22. Centrifuge at maximum speed for 1 minute.

23. Discard the filtrate and reuse the collection tube.

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

25. Centrifuge at maximum speed for 2 minutes to dry the column.

    Note: It is critical to remove all residual ethanol that might interfere with downstream applications.

26. Place the column into a 1.5 mL or 2.0 mL nuclease-free microcentrifuge tube.

27. Add 100 µL Elution Buffer heated to 70°C.

28. Let sit at room temperature for 3 minutes.

29. Centrifuge at maximum speed for 1 minute.

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

    Note: Incubation at 70°C will give a modest increase in DNA yield per elution. Using the first eluate for the second elution will increase DNA concentration.

31. Store DNA at -20°C.

Tip: Blood spots from finger pricks usually contain no more than 50 µL blood and yield approximately 500 ng to 1 µg DNA. This is sufficient for PCR analysis. To obtain higher DNA concentrations, elute with 50 µL heated Elution Buffer or TE and repeat the elution step with the first eluate.

E.Z.N.A.® Forensic DNA Kit Protocol - Fresh or Frozen Semen

This protocol can be used for fresh or frozen semen samples with equal efficiency. Frozen samples must to be completely thawed before use. Note that lysis time will vary depending on the size and density of the source material.

Materials and Reagents to be Supplied by User:

- 100% Ethanol
- Isopropanol
- 15 mL Corex glass centrifuge tubes
- Centrifuge capable of 2,500 x *g*
- Vortexer
- 2.0 mL nuclease-free microcentrifuge tubes
- Nuclease-free pipette tips
- Water bath or heat block capable of 60°C and 70°C
- NaCl, EDTA, Tris-HCl, SDS, β-mercaptoethanol to prepare Buffer A and Buffer B (see table below in the Before Starting section)
- Optional: 3M NaOH

Before Starting:

- Heat the water bath or heat block to 60°C
- Heat Elution Buffer to 70°C
- Prepare the DNA Wash Buffer and HBC Buffer according to the instructions in the Preparing Reagents section on Page 4
- Prepare the following buffers before beginning:

Buffer A:	150 mM NaCl 10 mM EDTA, pH 8.0	Buffer B:	100 mM Tris-HCl, pH 8.0 10 mM EDTA 500 mM NaCl 1% SDS 2% β-mercaptoethanol

1. Add 50-250 µL semen to 10 mL Buffer A in a 15 mL Corex centrifuge tube.

   Note: Using Corex tubes prevents attachment of the sperm cells to the tube walls.

2. Vortex for 10 seconds.

3. Centrifuge at 2,500 x *g* for 10 minutes.

4. Carefully aspirate the supernatant leaving ~1 mL pellet and Buffer A.

5. Vortex for 10 seconds.

6. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

7. Transfer sample to a new 2.0 mL microcentrifuge tube.

8. Add 500 µL Buffer A to the Corex tube from **Step 4** to rinse the tube.

9. Vortex for 30 seconds.

10. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

11. Transfer sample from **Step 8** to the 2 mL nuclease-free microcentrifuge tube.

12. Centrifuge at maximum speed for 2 minutes.

13. Carefully remove the supernatant without disturbing the semen pellet.

14. Add 200 µL Buffer B and resuspend pellet.

15. Add 50 µL OB Protease Solution.

16. Incubate at 60°C for 2 hours. Invert the tube occasionally to disperse the sample or place on a rocking platform.

17. Add 250 µL BL Buffer.

18. Add 260 µL 100% ethanol. Vortex to mix thoroughly.

19. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

20. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube provided with this kit.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit at room temperature 4 minutes.
3. Centrifuge at maximum speed for 30 seconds.
4. Discard the filtrate and reuse the collection tube.

21. Transfer the entire sample from **Step 19** to the column, including any precipitate that may have formed.

22. Centrifuge at maximum speed for 1 minute.

23. Discard the filtrate and the collection tube.

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

25. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

26. Centrifuge at maximum speed for 1 minute.

27. Discard the filtrate and the collection tube.

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

29. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see Page 4 for instructions.

30. Centrifuge at maximum speed for 1 minute.

31. Discard the filtrate and reuse the collection tube.

32. Repeat Steps 29-31 for a second DNA Wash step.

33. Centrifuge at maximum speed for 2 minutes to dry the column.

    Note: It is critical to remove all residual ethanol that might interfere with downstream applications.

34. Place the column into a 1.5 mL or 2.0 mL nuclease-free microcentrifuge tube.

35. Add 100 µL Elution Buffer heated to 70°C.

36. Let sit at room temperature for 3 minutes.

37. Centrifuge at maximum speed for 1 minute.

38. Repeat Steps 35-37 for a second elution step.

    Note: Incubation at 70°C will give a modest increase in DNA yield per elution. Using the first eluate for the second elution will increase DNA concentration.

39. Store DNA at -20°C.

E.Z.N.A.® Forensic DNA Kit Protocol - Buccal Swabs

This protocol has been tested for the following swab types: cotton and C.E.P. (Life Science). Typical yields from these swabs are 0.5-3 µg DNA.

Materials and Reagents to be Supplied by User:

- PBS
- 100% Ethanol
- Isopropanol
- 2 mL nuclease-free microcentrifuge tubes
- Nuclease-free pipette tips
- Microcentrifuge capable of 14,000 x *g*
- Vortexer
- Water baths or heat blocks capable of 60°C and 70°C
- Optional: 3M NaOH

Before Starting:

- Heat the Elution Buffer to 70°C
- Heat the water bath or heat block to 60°C
- Prepare the DNA Wash Buffer and HBC Buffer according to the instructions in the Preparing Reagents section on Page 4

1. Scrape the swabs firmly against the inside of each cheek 6 -7 times.

   Note: The person providing the sample should not eat or drink for at least 30 minutes prior to the sample collection.

2. Air or vacuum dry the swabs for 2 hours following collection.

3. Separate the swab from the stick and transfer to a 2 mL nuclease-free microcentrifuge tube.

4. Add 550 µL PBS.

5. Add 25 µL OB Protease Solution.

6. Add 550 µL BL Buffer. Vortex immediately for 30 seconds.

7. Incubate at 60°C for 30 minutes with occasional mixing.

8. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

9. Add 550 µL 100% ethanol. Vortex to mix thoroughly.

10. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

11. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube provided with this kit.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit at room temperature 4 minutes.
3. Centrifuge at maximum speed for 30 seconds.
4. Discard the filtrate and reuse the collection tube.

12. Carefully add 700 µL of the sample from **Step 10** to the HiBind® DNA Mini Column.

13. Centrifuge at maximum speed for 1 minute.

14. Discard the filtrate and reuse collection tube.

15. Repeat Steps 12-14 until all of the remaining sample has been transferred to the HiBind® DNA Mini Column.

16. Discard the collection tube.

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

18. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

19. Centrifuge at maximum speed for 1 minute.

20. Discard the filtrate and the collection tube.

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

22. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see Page 4 for instructions.

23. Centrifuge at maximum speed for 1 minute.

24. Discard the filtrate and reuse the collection tube.

25. Repeat Steps 22-24 for a second DNA Wash step.

26. Centrifuge at maximum speed for 2 minutes to dry the column.

    Note: It is critical to remove all residual ethanol that might interfere with downstream applications.

27. Transfer the HiBind® DNA Mini Column to a 2 mL nuclease-free microcentrifuge tube.

28. Add 200 µL Elution Buffer heated to 70°C.

29. Let sit at room temperature for 3 minutes.

30. Centrifuge at maximum speed for 1 minute.

31. Repeat Steps 31-33 for a second elution step.

    Note: Incubation at 70°C will give a modest increase in DNA yield per elution. Using the first eluate for the second elution will increase DNA concentration.

32. Store DNA at -20°C.

E.Z.N.A.® Forensic DNA Kit Protocol - Bacteria from Biological Fluids

Materials and Reagents to be Supplied by User:

- 100% Ethanol
- Isopropanol
- 1.5 mL or 2.0 mL nuclease-free microcentrifuge tubes
- Nuclease-free pipette tips
- Microcentrifuge capable of 14,000 x *g*
- Vortexer
- Water baths or heat blocks capable of 55°C, 60°C and 70°C
- Optional: 3M NaOH

Before Starting:

- Heat the water baths or heat blocks to 55°C and 60°C
- Heat the Elution Buffer to 70°C
- Prepare the DNA Wash Buffer and HBC Buffer according to the instructions in the Preparing Reagents section on Page 4

1. Centrifuge at 5,000 x *g* for 10 minutes to pellet bacteria.

2. Add 200 µL TL Buffer and resuspend bacterial pellet.

3. Incubate at 55°C for 15 minutes. Vortex every 2 minutes to mix.

4. Add 25 µL OB Protease Solution. Vortex to mix thoroughly.

5. Incubate at 60°C for 45 minutes with occasional mixing.

6. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

7. Add 225 µL BL Buffer. Vortex to mix thoroughly.

8. Incubate at 60°C for 10 minutes.

9. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

10. Add 300 µL isopropanol. Vortex to mix thoroughly.

11. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

12. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube provided with this kit.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit at room temperature 4 minutes.
3. Centrifuge at maximum speed for 30 seconds.
4. Discard the filtrate and reuse the collection tube.

13. Transfer the entire sample from **Step 11** to the column, including any precipitate that may have formed.

14. Centrifuge at maximum speed for 1 minute.

15. Discard the filtrate and the collection tube.

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

17. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

18. Centrifuge at maximum speed for 1 minute.

19. Discard the filtrate and the collection tube.

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 ethanol before use. Please see Page 4 for instructions.

22. Centrifuge at maximum speed for 1 minute.

23. Discard the filtrate and reuse the collection tube.

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

25. Centrifuge at maximum speed for 2 minutes to dry the column.

    Note: It is critical to remove all residual ethanol that might interfere with downstream applications.

26. Place the column into a 1.5 mL or 2.0 mL nuclease-free microcentrifuge tube.

27. Add 100 µL Elution Buffer heated to 70°C.

28. Let sit at room temperature for 3 minutes.

29. Centrifuge at maximum speed for 1 minute.

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

    Note: Incubation at 70°C will give a modest increase in DNA yield per elution. Using the first eluate for the second elution will increase DNA concentration.

31. Store DNA at -20°C.

E.Z.N.A.® Forensic DNA Kit Protocol - Saliva

Materials and Reagents to be Supplied by User:

- PBS
- 100% Ethanol
- Isopropanol
- Microcentrifuge capable of 14,000 x *g*
- Vortexer
- Water baths or heat blocks capable of 60°C and 70°C
- 15 mL centrifuge tubes
- 1.5 mL or 2.0 mL nuclease-free microcentrifuge tubes
- Nuclease-free pipette tips
- Optional: RNase A
- Optional: 3M NaOH

Before Starting:

- Heat the Elution Buffer to 70°C.
- Heat the water bath or heat block to 60°C.
- Prepare the DNA Wash Buffer and HBC Buffer according to the instructions in the Preparing Reagents section on Page 4

1. Collect 1.5 mL saliva in a 15 mL centrifuge tube containing 6 mL PBS. Vortex to mix thoroughly.

2. Centrifuge at 2,000 x *g* for 5 minutes.

3. Aspirate and discard the supernatant.

4. Add 180 µL PBS and resuspend the pellet.

5. Transfer the sample to a new 1.5 mL or 2.0 mL nuclease-free microcentrifuge tube.

   Note: If RNA-free DNA is desired, add 20 µL RNase A to the sample. Let sit at room temperature for 5 minutes.

6. Add 25 µL OB Protease Solution.

7. Add 200 µL BL Buffer. Vortex for 30 seconds to mix thoroughly.

8. Incubate at 60°C for 15 minutes with occasional mixing.

9. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

10. Add 200 µL 100% ethanol. Vortex to mix thoroughly.

11. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

12. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube provided with this kit.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit at room temperature 4 minutes.
3. Centrifuge at maximum speed for 30 seconds.
4. Discard the filtrate and reuse the collection tube.

13. Transfer the entire sample from **Step 11** to the column, including any precipitate that may have formed.

14. Centrifuge at maximum speed for 1 minute.

15. Discard the filtrate and the collection tube.

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

17. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

18. Centrifuge at maximum speed for 1 minute.

19. Discard the filtrate and the collection tube.

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 ethanol before use. Please see Page 4 for instructions.

22. Centrifuge at maximum speed for 1 minute.

23. Discard the filtrate and reuse the collection tube.

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

25. Centrifuge at maximum speed for 2 minutes to dry the column.

    Note: It is critical to remove all residual ethanol that might interfere with downstream applications.

26. Place the column into a 1.5 mL or 2.0 mL nuclease-free microcentrifuge tube.

27. Add 100 µL Elution Buffer heated to 70°C.

28. Let sit at room temperature for 3 minutes.

29. Centrifuge at maximum speed for 1 minute.

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

    Note: Incubation at 70°C will give a modest increase in DNA yield per elution. Using the first eluate for the second elution will increase DNA concentration.

31. Store DNA at -20°C.

E.Z.N.A.® Forensic DNA Kit Protocol - Hair, Nails, and Feathers

Materials and Reagents to be Supplied by User:

- PBS
- 100% Ethanol
- Isopropanol
- Microcentrifuge capable of 14,000 x *g*
- Vortexer
- Water baths or heat blocks capable of 60°C and 70°C
- 1.5 mL or 2.0 mL nuclease-free microcentrifuge tubes
- Nuclease-free pipette tips
- 1M DTT (dithiothreitol)
- Optional: 3M NaOH

Before Starting:

- Heat the water bath or heat block to 60°C
- Heat the Elution Buffer to 70°C
- Prepare the DNA Wash Buffer and HBC Buffer according to the instructions in the Preparing Reagents section on Page 4

1. Cut the sample into small pieces (0.5-1 cm) and transfer to a 1.5 mL nuclease-free microcentrifuge tube.

   Tip: For hair, cut from base of hair; for feathers, select the primary feathers. (Large birds, secondary tail, or breast feather can be use).

2. Add 250 µL TL Buffer.

3. Add 25 µL OB Protease Solution.

4. Add 20 µL 1M DTT. Vortex to mix thoroughly.

5. Incubate at 60°C for 30 minutes with occasional mixing.

6. Add 250 µL BL Buffer. Vortex to mix thoroughly.

7. Add 250 µL 100% ethanol. Vortex to mix thoroughly.

8. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

9. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube provided with this kit.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit at room temperature 4 minutes.
3. Centrifuge at maximum speed for 30 seconds.
4. Discard the filtrate and reuse the collection tube.

10. Transfer the entire sample from **Step 8** to the column, including any precipitate that may have formed.

11. Centrifuge at maximum speed for 1 minute.

12. Discard the filtrate and the collection tube.

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

14. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

15. Centrifuge at maximum speed for 1 minute.

16. Discard the filtrate and the collection tube.

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

18. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see Page 4 for instructions.

19. Centrifuge at maximum speed for 1 minute.

20. Discard the filtrate and reuse the collection tube.

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

22. Centrifuge at maximum speed for 2 minutes to dry the column.

    Note: It is critical to remove all residual ethanol that might interfere with downstream applications.

23. Place the column into a 1.5 mL or 2.0 mL nuclease-free microcentrifuge tube.

24. Add 100 µL Elution Buffer heated to 70°C.

25. Let sit at room temperature for 3 minutes.

26. Centrifuge at maximum speed for 1 minute.

27. Repeat Steps 24-26 for a second elution step.

    Note: Incubation at 70°C will give a modest increase in DNA yield per elution. Using the first eluate for the second elution will increase DNA concentration.

28. Store DNA at -20°C.

E.Z.N.A.® Forensic DNA Kit Protocol - Vacuum/Spin Protocol

Materials and Reagents to be Supplied by User:

- Vacuum manifold (Recommend Cat# VAC-08)
- Vacuum pump or vacuum source
- 100% Ethanol
- Isopropanol
- 1.5 mL or 2.0 mL nuclease-free microcentrifuge tubes
- Nuclease-free pipette tips
- Microcentrifuge capable of 14,000 x *g*
- Vortexer
- Water baths or heat blocks capable of 55°C, 60°C and 70°C
- Optional: 3M NaOH

Before Starting:

- Prepare the DNA Wash Buffer and HBC Buffer according to the instructions in the Preparing Reagents section on Page 4
- Heat the water baths or heat blocks to 55°C and 60°C
- Heat the Elution Buffer to 70°C

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

1. Cut or punch out the blood spot (or other sample) from the filter paper. (Up to 200 µL of blood can be used for each spot.) Tear or cut filter into small pieces and place into a microcentrifuge tube.

   Note: Use 3-4 punched circles (3 mm diameter) for each DNA isolation.

2. Add 200 µL TL Buffer. Vortex to mix thoroughly.

3. Incubate at 55°C for 15 minutes. Vortex every 2 minutes to mix.

4. Add 25 µL OB Protease Solution. Vortex to mix thoroughly.

5. Incubate at 60°C for 45 minutes with occasional mixing.

6. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

7. Add 225 µL BL Buffer. Vortex to mix thoroughly.

8. Incubate at 60°C for 10 minutes.

9. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

10. Add 300 µL isopropanol. Vortex to mix thoroughly.

11. Centrifuge at maximum speed to collect any sample adhering to the cap/walls of the tube.

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

13. Connect the HiBind® DNA Mini Column to the manifold.

Optional Protocol for Column Equilibration:

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

14. Transfer the sample from **Step 11** to the HiBind® DNA Mini Column.

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

16. Turn off the vacuum.

17. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

18. Turn on the vacuum source to draw the HB Buffer through the column.

19. Turn off the vacuum.

20. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see Page 4 for instructions.

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

22. Turn off the vacuum.

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

24. Transfer the HiBind® DNA Mini Column to a 2 mL Collection Tube provided with this kit.

25. Centrifuge at maximum speed for 2 minutes to dry the column.

    Note: It is critical to remove all residual ethanol that might interfere with downstream applications.

26. Transfer the HiBind® DNA Mini Column to a 1.5 mL nuclease-free microcentrifuge tube.

27. Add 30-50 µL Elution Buffer.

28. Let sit at room temperature for 1-2 minutes.

29. Centrifuge at 8,000 x *g* for 1 minute.

30. 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 **(800-832-8896)**.

Problem	Cause	Solution
Clogged Column	Incomplete lysis	Extend incubation time of lysis with TL Buffer and OB Protease. Add the correct volume of BL Buffer and incubate for specified time at 70°C. It may be necessary to extend incubation time by 10 minutes.
	Sample too large	If using more than 30 mg tissue, increase volumes of OB Protease, TL Buffer, BL Buffer, and ethanol. Pass aliquots of lysate through one column successively.
	Sample too viscous	Divide sample into multiple tubes, adjust volume to 250 µL with 10 mM Tris-HCl.
Low DNA Yield	Clogged column	See above
	Poor sample release from collection paper	Incubate the specimen collection paper longer in TL Buffer. Shake the tubes frequently.
	Poor elution	Repeat elution or increase elution volume (see note on Page 4). Incubation of column at 70°C for 5 minutes with Elution Buffer may increase yields.
	Improper washing	DNA Wash Buffer must be diluted with ethanol as specified on Page 4 before use.
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation. It will not interfere with PCR or restriction digests.
	Poor cell lysis due to incomplete mixing with BL Buffer	Repeat the procedure. Make sure to vortex the sample thoroughly with BL Buffer immediately.
	Incomplete cell lysis or protein degradation due to insufficient incubation	Increase incubation time with TL Buffer and OB Protease. Ensure that no visible pieces of tissue remain.
	Samples are rich in protein	After applying to column, wash with 300 µL of a 1:1 mixture of BL Buffer and ethanol and then wash with DNA Wash Buffer.
No DNA Eluted	Poor cell lysis due to improper mixing with BL Buffer	Mix thoroughly with BL Buffer prior to loading the HiBind® DNA Mini Column.
	Poor cell and/or protein lysis in TL Buffer	Tissue sample must be cut or minced into small pieces. Increase incubation time at 65°C with TL Buffer to ensure that tissue is completely lysed.
	Ethanol not added to BL Buffer	Before applying sample to column, an aliquot of BL Buffer/ethanol must be added. See protocol above.
	No ethanol added to the DNA Wash Buffer	Dilute DNA Wash Buffer with the indicated volume of ethanol before use.
	No isopropanol added to the HBC Buffer	Dilute HBC Buffer with the indicated volume of isopropanol before use.
Colored residue in column	Incomplete lysis due to improper mixing with BL Buffer	BL Buffer is viscous and the sample must be vortexed thoroughly.
	Ethanol was not added to DNA Wash Buffer	Dilute DNA Wash Buffer with the indicated volume of ethanol before use.

Ordering Information

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

Buffer (Size)	Part Number
BL Buffer, 100 mL	PD062
TL Buffer, 100 mL	PD061
DNA Wash Buffer, 100 mL	PS010
Elution Buffer, 100 mL	PDR048