OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Tissue DNA Kit

D3396-00	5 preps
D3396-01	50 preps
D3396-02	200 preps

May 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® Tissue DNA Kit

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Manual Revision: May 2013

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Introduction and Overview

The E.Z.N.A.® family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is the new HiBind matrix that specifically, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or a low salt buffer.

The E.Z.N.A.® Tissue DNA Kit provides an easy and rapid method for the isolation of genomic DNA for consistent PCR and Southern analysis. Up to 30 mg animal tissue, mouse tail snips, paraffin-embedded tissue, or 5 x 10^6 cultured cells can be readily processed. This kit allows for the single or multiple simultaneous processing of samples. There is no need for phenol/chloroform extractions and time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA can be directly used for most applications such as PCR, Southern blotting, and restriction enzyme digestion.

Benefits of the E.Z.N.A.® Tissue DNA Kit

- Optimized buffers that guarantee pure DNA
- No organic extractions
- Purified DNA can be directly used for most downstream applications

New in this Edition

- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer (used in the Troubleshooting section) is no longer included with this kit.
- Equilibration Buffer can be replaced with 3M NaOH provided by the user.
- The total number of 2 mL Collection Tubes has been reduced from 600 to 400. This change eliminates a transfer step and reduces waste.
- OB Protease is now supplied in a liquid form eliminating the resuspension step to prior to use.
- OB Protease Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Yield and Quality of DNA

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

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

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

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

Expected Yields

Source	Sample Amount	Yield (µg)
Whole Blood	200 µL	4-12 µg
Mouse Tail	20 mg	15-25 µg
HeLa Cells	1 x 10^6 cells	5-6 µg
Liver	20 mg	13-22 µg

Illustrated Protocol

Kit Contents

Product	D3396-00	D3396-01	D3396-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
BL Buffer	5 mL	20 mL	60 mL
TL Buffer	5 mL	20 mL	60 mL
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	1.5 mL	15 mL	3 x 25 mL
Elution Buffer	2 mL	30 mL	2 x 50 mL
OB Protease Solution	150 µL	1.5 mL	6.0 mL
User Manual	✓	✓	✓

Storage and Stability

All E.Z.N.A.® Tissue DNA Kit components are guaranteed for at least 12 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

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

Kit	100% Ethanol to be Added
D3396-00	6 mL
D3396-01	60 mL
D3396-02	100 mL per bottle

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

Kit	Isopropanol to be Added
D3396-00	1.6 mL
D3396-01	10 mL
D3396-02	32 mL

- Check buffers for precipitation before use. Redissolve any precipitates by warming to 37˚C.

Recommended Settings

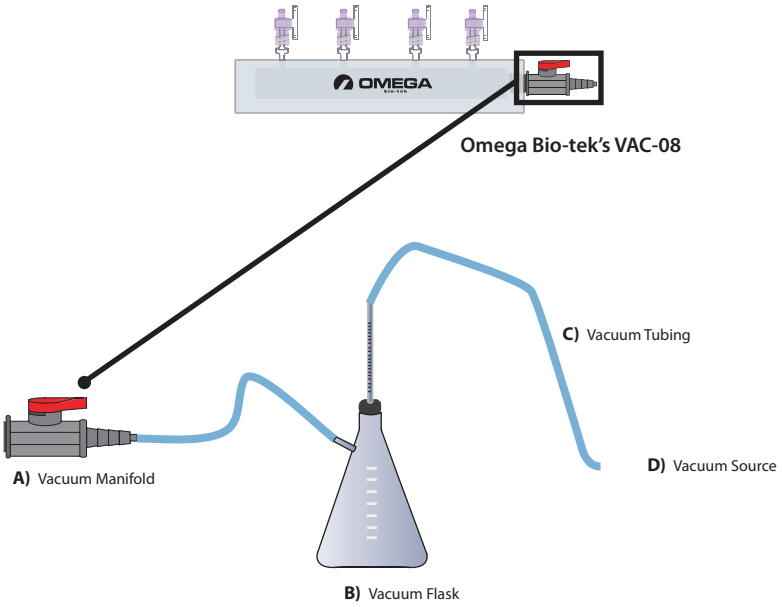
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, 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 (mm Hg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup:



E.Z.N.A.® Tissue DNA Kit Protocol - Tissue

This method is suitable for the isolation of DNA from up to 30 mg tissue. Yields vary depending on source.

Optional: Although no mechanical homogenization of tissue is necessary, pulverizing the samples in liquid nitrogen will improve lysis and reduce incubation time. Once the liquid nitrogen has evaporated, transfer the powdered tissue into a clean 1.5 mL microcentrifuge tube. Add 200 µL TL Buffer and proceed to Step 2 below.

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Shaking water baths, heat blocks, or incubators capable of 55-70°C
- Vortexer
- 100% ethanol
- Isopropanol
- Optional: RNase stock solution (100 mg/mL)

Before Starting:

- Set water baths, heat blocks, or incubators to 55°C and 70°C.
- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 6.
- Heat Elution Buffer to 70°C.

1. Mince up to 30 mg tissue and transfer in a 1.5 mL microcentrifuge tube.

2. Add 200 µL TL Buffer.

   Note: In order to speed up lysis, cut the tissue into small pieces. For samples more than 30 mg, simply scale up the volume of TL Buffer used; for a 40-60 mg sample use 400 µL TL Buffer.

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

4. Incubate at 55°C in a shaking water bath.

   Note: If a shaking water bath is not available, vortex the sample every 20-30 minutes. Lysis time depends on the amount and type of tissue used. The average time is usually less than 3 hours. Lysis can proceed overnight.

Optional: Certain tissues such as liver tissue have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

1. Add 4 µL RNase A (100 mg/mL) per 30 mg tissue.
2. Let sit at room temperature for 2 minutes.
3. Proceed to Step 5 below.

5. Centrifuge at maximum speed (≥10,000 x *g*) for 5 minutes.

6. Transfer the supernatant to a sterile 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet.

7. Add 220 µL BL Buffer. Adjust the volume of BL Buffer based on the amount of starting material. Vortex to mix thoroughly.

   Example: If you used 400 µL of TL Buffer then add 420 µL BL Buffer and 420 µL 100% ethanol.

   Note: A wispy precipitate may form upon the addition of BL Buffer. This is does not interfere with DNA recovery.

8. Incubate at 70°C for 10 minutes.

9. Add 220 µL 100% ethanol. Adjust the volume of ethanol required based on the amount of starting material. Vortex to mix thoroughly.

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

11. Transfer the entire sample from Step 9 to the HiBind® DNA Mini Column including any precipitates that may have formed.

12. Centrifuge at maximum speed for 1 minute.

13. Discard the filtrate and reuse the collection tube.

14. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

15. Centrifuge at maximum speed for 30 seconds.

16. Discard the filtrate and collection tube.

17. Insert the HiBind® DNA Mini Column into 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 6 for instructions.

19. Centrifuge at maximum speed for 30 seconds.

20. Discard the filtrate and reuse the collection tube.

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

22. 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.

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

24. Add 100-200 µL Elution Buffer heated to 70°C.

25. Let sit at room temperature for 2 minutes.

26. Centrifuge at maximum speed for 1 minute.

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

    Note: Each 200 µL elution 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. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

28. Store eluted DNA at -20°C.

E.Z.N.A.® Tissue DNA Kit Protocol - Cultured Cells

This protocol is designed for the rapid isolation of up to 25 µg genomic DNA from up to 5 x 10^6 cultured cells.

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Shaking water bath, heat block, or incubator capable of 70°C
- Vortexer
- 100% ethanol
- Isopropanol
- PBS
- Optional: RNase stock solution (100 mg/mL)

Before Starting:

- Set water bath, heat block, or incubator to 70°C
- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 6.
- Heat Elution Buffer to 70°C
- Chill PBS to 4°C.

1. Prepare the cell suspension using one of the following methods:

   A) Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation. Wash the cells with cold PBS (4°C). Resuspend cells in 200 µL PBS. Proceed to Step 2.

   B) For cells grown in suspension, pellet 5 x 10^6 by spinning at 1,200 x *g* in a centrifuge tube. Aspirate and discard the supernatant, and wash the cells once with cold PBS (4°C). Resuspend cells in 200 µL PBS. Proceed to Step 2.

   C) For cells grown in a monolayer, harvest the cell by either using a trypsin treatment or by scraping with a rubber policeman. Wash cells twice with cold PBS (4°C). Resuspend the cells in 200 µL PBS. Proceed to Step 2.

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

Optional: Cultured cells have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

1. Add 4 µL RNase A (100 mg/mL) per 30 mg tissue.
2. Let sit at room temperature for 2 minutes.
3. Proceed to Step 3 below.

3. Add 220 µL BL Buffer.

   Note: A wispy precipitate may form upon the addition of BL Buffer. This is does not interfere with DNA recovery.

4. Incubate at 70°C for 10 minutes. Briefly vortex the tube once during incubation.

5. Add 220 µL 100% ethanol. Adjust the volume of ethanol required based on the amount of starting material. Vortex to mix thoroughly.

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

7. Transfer the entire sample from Step 5 to the HiBind® DNA Mini Column including any precipitates that may have formed.

8. Centrifuge at maximum speed (≥10,000 x *g*) for 1 minute.

9. Discard the filtrate and reuse the collection tube.

10. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

11. Centrifuge at maximum speed for 30 seconds.

12. Discard the filtrate and collection tube.

13. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

14. Add 700 µL DNA Wash Buffer.

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

15. Centrifuge at maximum speed for 30 seconds.

16. Discard the filtrate and reuse the collection tube.

17. Repeat Steps 14-16 for a second DNA Wash Buffer wash step.

18. 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**.**

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

20. Add 100-200 µL Elution Buffer heated to 70°C.

21. Let sit at room temperature for 2 minutes.

22. Centrifuge at maximum speed for 1 minute.

23. Repeat Steps 20-22 for a second elution step.

    Note: Each 200 µL elution 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. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

24. Store eluted DNA at -20°C.

E.Z.N.A.® Tissue DNA Kit Protocol - Mouse Tail Snips

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Shaking water baths, heat blocks, or incubators capable of 55-70°C
- Vortexer
- 100% ethanol
- Isopropanol
- Optional: RNase stock solution (100 mg/mL)

Before Starting:

- Set water baths, heat blocks, or incubators to 55°C and 70°C.
- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 6.
- Heat Elution Buffer to 70°C.

1. Snip two pieces of mouse tail 0.2-0.5 cm in length and place into a nuclease-free 1.5 mL microcentrifuge tube

   Note: Follow all regulations regarding the safe and humane treatment of animals. Mice should not be older than 6 weeks as lysis will be more difficult in older animals resulting in suboptimal DNA yields. If possible, obtain tail biopsies at 2-4 weeks and freeze samples at -70°C until DNA is extracted.

2. Add 200 µL TL Buffer.

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

4. Incubate at 55°C for 1-4 hours in a shaking water bath.

   Note: If a shaking water bath is not available, vortex the sample every 20-30 minutes. Lysis time depends on the amount and type of tissue used. Incubation time for complete tail lysis is dependent on tail length, and animal age; 0.5 cm tail pieces from a two-week old mice will typically lyse in approximately 2 hours. For older animals, an overnight incubation may improve yields. Bone and hair will not lyse.

Mouse Tail Snips Protocol

Optional: Mouse tail snips have low levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

1. Add 4 µL RNase A (100 mg/mL) per 30 mg tissue.
2. Let sit at room temperature for 2 minutes.
3. Proceed to Step 5 below.

5. Centrifuge at maximum speed (≥10,000 x *g*) for 5 minutes to pellet insoluble tissue debris and hair.

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

7. Add one volume BL Buffer and one volume 100% ethanol. Vortex to mix thoroughly.

   Example: If you transfer 180 µL cleared lysate, add 180 µL BL Buffer and 180 µL 100% ethanol.

   Note: A wispy precipitate may form upon the addition of BL Buffer. This is does not interfere with DNA recovery.

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

9. Transfer the entire sample from Step 7 to the HiBind® DNA Mini Column including any precipitates that may have formed.

10. Centrifuge at maximum speed for 1 minute.

11. Discard the filtrate and reuse the collection tube.

12. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

13. Centrifuge at maximum speed for 30 seconds.

14. Discard the filtrate and collection tube.

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

16. Add 700 µL DNA Wash Buffer.

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

17. Centrifuge at maximum speed for 30 seconds.

18. Discard the filtrate and reuse the collection tube.

19. Repeat Steps 16-18 for a second DNA Wash Buffer wash step.

20. 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.

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

22. Add 100-200 µL Elution Buffer heated to 70°C.

23. Let sit at room temperature for 2 minutes.

24. Centrifuge at maximum speed for 1 minute.

25. Repeat Steps 22-24 for a second elution step.

    Note: Each 200 µL elution 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. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

26. Store eluted DNA at -20°C.

E.Z.N.A.® Tissue DNA Kit Protocol - Paraffin-embedded Tissue

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Shaking water baths, heat blocks, or incubators capable of 37-90°C
- Vortexer
- Incubator
- 100% ethanol
- Isopropanol
- Xylene
- Optional: RNase stock solution (100 mg/mL)

Before Starting:

- Set water baths, heat blocks, or incubators to 37°C, 55°C, 70°C , and 90°C.
- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 6.
- Heat Elution Buffer to 70°C.

1. Place no more than 30 mg of tissue (~2 mm^3) in a nuclease-free 2 mL microcentrifuge tube.

2. Add 1 mL xylene. Vortex to mix thoroughly.

3. Centrifuge at maximum speed (≥10,000 x *g*) for 10 minutes.

4. Aspirate and discard the supernatant without disturbing the pellet.

5. Add 1 mL 100% ethanol.

6. Centrifuge at maximum speed for 5 minutes.

7. Aspirate and discard the ethanol without disturbing the pellet.

8. Repeat Steps 5-7 for a second ethanol wash step.

9. Dry the tissue pellet at 37°C for 15 minutes.

10. Add 200 µL TL Buffer.

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

12. Incubate at 55°C in a shaking water bath.

    Note: If a shaking water bath is not available, vortex the sample every 20-30 minutes. Lysis time depends on the amount and type of tissue used. The average time is usually less than 3 hours. Lysis can proceed overnight.

13. Incubate at 90°C for 30-60 minutes.

Optional: Certain tissues such as liver tissue have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

1. Add 4 µL RNase A (100 mg/mL) per 30 mg tissue.
2. Let sit at room temperature for 2 minutes.
3. Proceed to Step 14 below.

14. Centrifuge at maximum speed for 5 minutes.

15. Transfer the supernatant to a sterile 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet.

16. Add 220 µL BL Buffer. Adjust the volume of BL Buffer based on the amount of starting material. Vortex to mix thoroughly.

    Note: A wispy precipitate may form upon the addition of BL Buffer. This is does not interfere with DNA recovery.

17. Incubate at 70°C for 10 minutes.

18. Add 220 µL 100% ethanol. Adjust the volume of ethanol required based on the amount of starting material. Vortex to mix thoroughly.

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

20. Transfer the entire sample from Step 18 to the HiBind® DNA Mini Column including any precipitates that may have formed.

21. Centrifuge at maximum speed for 1 minute.

22. Discard the filtrate and reuse the collection tube.

23. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

24. Centrifuge at maximum speed for 30 seconds.

25. Discard the filtrate and collection tube.

26. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

27. Add 700 µL DNA Wash Buffer.

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

28. Centrifuge at maximum speed for 30 seconds.

29. Discard the filtrate and reuse the collection tube.

30. Repeat Steps 27-29 for a second DNA Wash Buffer wash step.

31. 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**.**

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

33. Add 50-100 µL Elution Buffer heated to 70°C.

34. Let sit at room temperature for 2 minutes.

35. Centrifuge at maximum speed for 1 minute.

36. Repeat Steps 33-35 for a second elution step.

    Note: Yields will depend on size and age of sample. Certain samples may require prolonged lysis with TL Buffer. Tissue fixed with paraformaldehyde will yield degraded DNA or RNA. The extent of degradation depends on type of fixative used but the size of DNA obtained is usually less than 500 bp. Degradation is not caused by the E.Z.N.A.™ Tissue DNA Protocol.

37. Store eluted DNA at -20°C.

E.Z.N.A.® Tissue DNA Kit Protocol - Whole Blood and Body Fluids

The procedure below has been optimized for the use with fresh or frozen blood samples of 11-250 µL in volume. Anti-coagulated blood, saliva, serum, buffy coat, or other body fluids also can be used.

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Shaking water baths, heat blocks, or incubators capable of 70°C
- Vortexer
- 100% ethanol
- Isopropanol
- Optional: PBS
- Optional: 10 mM Tris-HCl
- Optional: RNase stock solution (100 mg/mL)

Before Starting:

- Set water bath, heat block, or incubator to 70°C.
- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 6.
- Heat Elution Buffer to 70°C.

1. Transfer the sample into a nuclease-free 1.5 mL microcentrifuge tube and bring the volume up to 250 µL with 10 mM Tris-HCl, PBS, or Elution Buffer (provided).

2. Add 25 µL OB Protease Solution.

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

   Note: A wispy precipitate may form upon the addition of BL Buffer. This is does not interfere with DNA recovery.

Optional: RNA will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

1. Add 4 µL RNase A (100 mg/mL).
2. Let sit at room temperature for 2 minutes.
3. Proceed to Step 4 below.

4. Incubate at 70°C for 10 minutes. Briefly vortex the tube once during incubation.

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

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

7. Transfer the entire sample from Step 5 to the HiBind® DNA Mini Column including any precipitates that may have formed.

8. Centrifuge at maximum speed (≥10,000 x *g*) for 1 minute.

9. Discard the filtrate and reuse the collection tube.

10. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

11. Centrifuge at maximum speed for 30 seconds.

12. Discard the filtrate and collection tube.

13. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

14. Add 700 µL DNA Wash Buffer.

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

15. Centrifuge at maximum speed for 30 seconds.

16. Discard the filtrate and reuse the collection tube.

17. Repeat Steps 14-16 for a second DNA Wash Buffer wash step.

18. 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**.**

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

20. Add 50-200 µL Elution Buffer heated to 70°C.

21. Let sit at room temperature for 2 minutes.

22. Centrifuge at maximum speed for 1 minute.

23. Repeat Steps 20-22 for a second elution step.

    Note: Each 200 µL elution 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. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

24. Store eluted DNA at -20°C.

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

Carry out disruption, homogenization, protease digestion, and loading onto the HiBind® DNA Mini Column as indicated in previous protocols. Instead of continuing with centrifugation, follow the steps outlined below.

Note: Please read through previous sections of this manual before beginning this protocol paying particular attention to the "Materials and Equipment to be Supplied by User".

Materials and Equipment to be Supplied by User:

- Vacuum Manifold (recommend Cat# VAC-08)
- Tabletop microcentrifuge capable of 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Shaking water bath, heat block, or incubator capable of 70°C
- Vortexer
- 100% ethanol
- Isopropanol

Before Starting:

- Set water bath, heat block, or incubator to 70°C.
- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 6.
- Heat Elution Buffer to 70°C.

1. Prepare samples by following one of the protocols above:
    1. Tissue Protocol Page 8, Steps 1-9
    2. Cultured Cells Protocol Page 12, Steps 1-5
    3. Mouse Tail Snips Page 16, Steps 1-7
    4. Paraffin-embedded Tissue Page 20, Steps 1-18
    5. Whole Blood and Body Fluids Page 24, Steps 1-5

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

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

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

5. Turn off the vacuum.

6. Add 500 µL HBC Buffer.

   Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

7. Switch on vacuum source to draw the HB Buffer through the column.

8. Turn off the vacuum.

9. Add 700 µL DNA Wash Buffer.

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

10. Switch on vacuum source to draw the DNA Wash Buffer through the column.

11. Turn off the vacuum.

12. Repeat Steps 9-11 for a second DNA Wash step.

13. Remove the column from the vacuum manifold and transfer to a new 2 mL Collection Tube.

14. Centrifuge at maximum speed (≥10,000 x g) for 2 minutes to completely dry the membrane.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

15. Insert the HiBind® DNA Mini Column into a new nuclease-free 1.5 mL microcentrifuge tube.

16. Add 50-200 µL Elution Buffer heated to 70°C.

    Note: Refer to individual protocols for recommended elution volumes.

17. Let sit at room temperature for 2 minutes.

18. Centrifuge at maximum speed for 1 minute.

19. Repeat Steps 16-18 for a second elution step.

    Note: Each 200 µL elution 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. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

20. 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 **1-800-832-8896**.

Problem	Cause	Solution
Clogged Column	Incomplete lysis	Extend lysis time with TL Buffer and OB Protease Solution.
	Sample size is too large	If using more than 30 mg tissue, increase volumes of OB Protease, TL Buffer, BL Buffer, and ethanol.
	Sample is viscious	Divide sample into multiple tubes and adjust the volume to 250 µL with TL Buffer.
Problem	Cause	Solution
Low DNA Yield	Incomplete homogenization	Completely homogenize sample.
	Poor elution	Repeat elution with increased elution volume. Incubate columns at 70°C for 5 minutes with Elution Buffer.
	Improper washing	• DNA Wash Buffer must be diluted with 100% ethanol before use. • HBC Buffer must be diluted with isopropanol before use.
	Overgrown culture	Overgrown culture contains lysed cells and degraded DNA.
	Sample has low DNA content	Increase starting material and volume of all reagents (OB Protease, TL Buffer, BL Buffer, ethanol) proportionally. Load aliquots of lysate through the column successively.
	Column matrix lost binding capacity during storage	Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x *g* for 30 seconds. Add 100 µL water to the columns and centrifuge at 10,000 x *g* for 30 seconds. Discard the filtrate.
Problem	Cause	Solution
Low A_{260}/A_{280} Ratio	Extended centrifugation during elution	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be remove 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.

Ordering Information

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

Product	Part Number
Vacuum Manifold	VAC-08
DNase/RNase-free microcentrifuge tubes, 1.5 mL, 500/pk, 10 pk/cs	SSI-1210-00
DNase/RNase-free microcentrifuge tubes, 2.0 mL, 500/pk, 10 pk/cs	SSI-1310-00
HiBind® DNA Mini Columns (200 columns)	DNACOL-02
BL Buffer (100 mL)	PD062
TL Buffer (100 mL)	PD061
DNA Wash Buffer (100 mL)	PS010
Elution Buffer (100 mL)	PDR048
OB Protease Solution (2 mL)	AC130
OB Protease Solution (10 mL)	AC131

Notes: