

E.Z.N.A.[®] Tissue DNA Kit

D3396-00	5 preps
D3396-01	50 preps
D3396-02	200 preps
D3396-03	600 preps

Manual Date: April 2024
Revision Number: v9.2

For Research Use Only

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E.Z.N.A.® Tissue DNA Kit

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Introduction

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 5×10^6 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

April 2024

- Addition of Warnings and Safety Information.

March 2022

- A new 600 prep kit has been added and is now available for purchase.

March 2019

- OB Protease Solution has been renamed Proteinase K Solution. This is a name change only. The formulation has not changed.

August 2018

- AC130 and AC131 have been discontinued. If additional OB Protease Solution is required, please call toll free (1-800-832-8896) to speak with a Customer Service representative.

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:

$$\text{DNA concentration} = \text{Absorbance 260} \times 50 \times (\text{Dilution Factor}) \mu\text{g/mL}$$

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

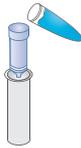
Illustrated Protocol



Lyse



Adjust Binding
Conditions



Bind



Wash 3X



Dry



Elute

Kit Contents

Product	D3396-00	D3396-01	D3396-02	D3396-03
Purifications	5	50	200	600
HiBind® DNA Mini Columns	5	50	200	600
2 mL Collection Tubes	10	100	400	1200
BL Buffer	5 mL	20 mL	60 mL	2 x 100 mL
TL Buffer	5 mL	20 mL	60 mL	135 mL
HBC Buffer	5 mL	25 mL	80 mL	250 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL	200 mL
Elution Buffer	15 mL	30 mL	2 x 60 mL	300 mL
Proteinase K Solution	150 µL	1.5 mL	6 mL	18 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: Proteinase K Solution can be stored at room temperature for up to 6 months from receipt. For long-term storage of Proteinase K Solution (>6 months), store at 2-8°C. Store all other components 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.

Warnings and Safety Information

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.

Preparing Reagents

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

Kit	100% Ethanol to be Added
D3396-00	10 mL
D3396-01	100 mL
D3396-02	100 mL per bottle
D3396-03	800 mL

- Dilute HBC Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
D3396-00	2 mL
D3396-01	10 mL
D3396-02	32 mL
D3396-03	100 mL

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

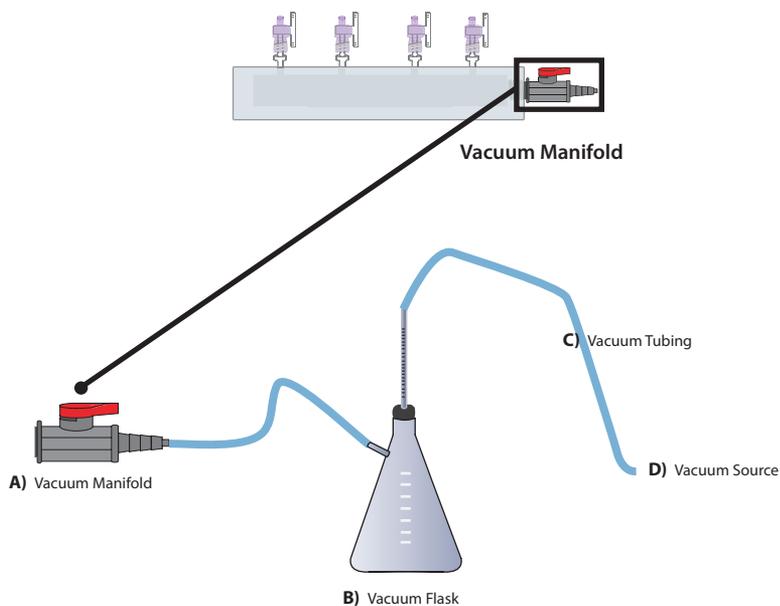
Recommended Settings

The following is required for use with the Vacuum Protocol:

- A) Vacuum Manifold
 - Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
- B) Vacuum Flask
- C) Vacuum Tubing
- D) Vacuum Source (review tables below for pressure settings)

Conversion from millibars:	Multiply by:
Millimeters of mercury (mm Hg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torr (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 Protocol

This method is suitable for the isolation of DNA from up to 30 mg tissue. Yields vary depending on source. The protocol can be scaled up to accommodate larger tissue samples, but additional TL Buffer and BL Buffer will need to be purchased separately.

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 μ L TL Buffer and proceed to Step 2 below.

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of 13,000g
- Shaking water baths, heat blocks, or incubators capable of 55-70°C
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- 100% 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 7.
- 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 μ L 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 μ L TL Buffer.

3. Add 25 μ L Proteinase K Solution. Vortex to mix thoroughly.

E.Z.N.A.[®] Tissue DNA Kit Protocol

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 copurified 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 ($\geq 10,000g$) 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.

Note: A wispy precipitate may form upon the addition of BL Buffer. This 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.

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

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

E.Z.N.A.[®] Tissue DNA Kit Protocol

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 μ L HBC Buffer.

Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 7 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 μ L DNA Wash Buffer.

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

E.Z.N.A.[®] Tissue DNA Kit Protocol

23. Transfer the HiBind[®] DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.
24. Add 100-200 μ L 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 μ L 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 μ L Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 μ L 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 Protocol

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

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of 13,000g
- Shaking water bath, heat block, or incubator capable of 70°C
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- 100% 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 7.
- 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 5×10^6 by spinning at 1,200g 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.

E.Z.N.A.[®] Tissue DNA Kit Protocol

2. Add 25 μ L Proteinase K Solution. Vortex to mix thoroughly.

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

1. Add 4 μ L 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 μ L BL Buffer.

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

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

5. Add 220 μ L 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 ($\geq 10,000g$) for 1 minute.

9. Discard the filtrate and reuse the collection tube.

10. Add 500 μ L HBC Buffer.

Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 7 for instructions.

E.Z.N.A.[®] Tissue DNA Kit Protocol

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 μ L DNA Wash Buffer.
Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 7 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 μ L Elution Buffer heated to 70°C.
21. Let sit at room temperature for 2 minutes.
22. Centrifuge at maximum speed for 1 minute.

E.Z.N.A.[®] Tissue DNA Kit Protocol

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

Note: Each 200 μ L 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 μ L Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 μ L 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 Protocol

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of 13,000g
- Shaking water baths, heat blocks, or incubators capable of 55-70°C
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- 100% 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 7.
- 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 Proteinase K Solution. Vortex to mix thoroughly.

E.Z.N.A.[®] Tissue DNA Kit Protocol

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.

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

1. Add 4 μ L 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 ($\geq 10,000g$) 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 μ L cleared lysate, add 180 μ L BL Buffer and 180 μ L 100% ethanol.

Note: A wispy precipitate may form upon the addition of BL Buffer. This 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.

E.Z.N.A.[®] Tissue DNA Kit Protocol

11. Discard the filtrate and reuse the collection tube.

12. Add 500 μ L HBC Buffer.

Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 7 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 μ L DNA Wash Buffer.

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

E.Z.N.A.[®] Tissue DNA Kit Protocol

21. Transfer the HiBind[®] DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.
22. Add 100-200 μ L 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 μ L 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 μ L Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 μ L 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 Protocol

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge capable of 13,000g
- Shaking water baths, heat blocks, or incubators capable of 37-90°C
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- Incubator
- 100% ethanol
- 100% 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 7.
 - Heat Elution Buffer to 70°C.
1. Place no more than 30 mg of tissue (~2 mm³) in a nuclease-free 2 mL microcentrifuge tube.
 2. Add 1 mL xylene. Vortex to mix thoroughly.
 3. Centrifuge at maximum speed (≥10,000g) 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.

E.Z.N.A.[®] Tissue DNA Kit Protocol

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 μ L TL Buffer.
11. Add 25 μ L Proteinase K 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 copurified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

1. Add 4 μ L 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 μ L 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 does not interfere with DNA recovery.

E.Z.N.A.[®] Tissue DNA Kit Protocol

17. Incubate at 70°C for 10 minutes.
18. Add 220 μ L 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 μ L HBC Buffer.
Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 7 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 μ L DNA Wash Buffer.
Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 7 for instructions.
28. Centrifuge at maximum speed for 30 seconds.

E.Z.N.A.[®] Tissue DNA Kit Protocol

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 μ L 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 Protocol

The procedure below has been optimized for the use with fresh or frozen blood samples of 11-250 μ L 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 13,000g
- Shaking water baths, heat blocks, or incubators capable of 70°C
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- 100% 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 7.
 - 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 μ L with 10 mM Tris-HCl, PBS, or Elution Buffer (provided).
 2. Add 25 μ L Proteinase K Solution.

E.Z.N.A.[®] Tissue DNA Kit Protocol

3. Add 250 μ L BL Buffer. Vortex to mix thoroughly.

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

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

1. Add 4 μ L 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 μ L 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 ($\geq 10,000g$) for 1 minute.
 9. Discard the filtrate and reuse the collection tube.
 10. Add 500 μ L HBC Buffer.

Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 7 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.

E.Z.N.A.® Tissue DNA Kit Protocol

14. Add 700 µL DNA Wash Buffer.

Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 7 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
- Tabletop microcentrifuge capable of 13,000g
- Shaking water bath, heat block, or incubator capable of 70°C
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- 100% 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 7.
- Heat Elution Buffer to 70°C.

1. Prepare samples by following one of the protocols above:
 1. Tissue Protocol Page 9, Steps 1-9
 2. Cultured Cells Protocol Page 13, Steps 1-5
 3. Mouse Tail Snips Page 17, Steps 1-7
 4. Paraffin-embedded Tissue Page 21, Steps 1-18
 5. Whole Blood and Body Fluids Page 25, 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.

E.Z.N.A.[®] Tissue DNA Kit Protocol

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

5. Turn off the vacuum.

6. Add 500 μ L HBC Buffer.

Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 7 for instructions.

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

8. Turn off the vacuum.

9. Add 700 μ L DNA Wash Buffer.

Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 7 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 ($\geq 10,000g$) 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.

E.Z.N.A.[®] Tissue DNA Kit Protocol

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

16. Add 50-200 μ L 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 μ L 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 μ L Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 μ L 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 Proteinase K Solution.
	Sample size is too large	If using more than 30 mg tissue, increase volumes of Proteinase K Solution, TL Buffer, BL Buffer, and ethanol.
	Sample is viscous	Divide sample into multiple tubes and adjust the volume to 250 μ L with TL Buffer.
Problem	Cause	Solution
Low DNA Yield	Incomplete homogenization	Completely homogenize the sample.
	Poor elution	Repeat elution with increased elution volume. Incubate columns at 70°C for 5 minutes with Elution Buffer.
	Improper washing	<ul style="list-style-type: none"> • DNA Wash Buffer must be diluted with 100% ethanol before use. • HBC Buffer must be diluted with 100% 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 (Proteinase K Solution, TL Buffer, BL Buffer, ethanol) proportionally. Load aliquots of lysate through the column successively.
	Column matrix lost binding capacity during storage	Add 100 μ L 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000g for 30 seconds. Add 100 μ L water to the columns and centrifuge at 10,000g 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 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.

Notes:

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