Genesee Scientific
A Life Science Company

Product Manual

GENE CHOICE
A Genesee Scientific Brand

Cell & Tissue DNA Miniprep Kit

Table of Contents

Introduction

The Gene Choice® Cell & Tissue DNA Purification Kit 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 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 Gene Choice® Cell & Tissue DNA Purification 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 Gene Choice® Cell & Tissue DNA Purification Kit

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

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:

$$DNA\ concentration = Absorbance\ 260 \times 50 \times (Dilution\ Factor)\ \mu g/ml$$

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 ml	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

Genesee Scientific
A Life Science Company

CALL: 800.789.5550 • **FAX:** 888.789.0444 • **WEB:** www.geneseesci.com • **EMAIL:** support@geneseesci.com

Kit Contents/Storage and Stability

Kit Contents

Product	SMP96-317	96-317	96-317B
Purifications	5	50	200
DNA Mini Columns	5	50	200
2 ml Collection Tubes	10	100	400
LB2 Buffer	5 ml	20 ml	60 ml
LB1 Buffer	5 ml	20 ml	60 ml
LB3 Buffer	5 ml	25 ml	80 ml
DNA Wash Buffer	2.5 ml	25 ml	3 x 25 ml
Elution Buffer	15 ml	30 ml	2 x 60 ml
Proteinase K Solution	150 µl	1.5 ml	6 ml
User Manual	✓	✓	✓

Storage and Stability

All 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 (>6 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
SMP96-317	10 ml
96-317	100 ml
96-317B	100 ml per bottle

• Dilute LB3 Buffer with 100% isopropanol as follows and store at room temperature

Kit	100% Isopropanol to be Added
SMP96-317	2 ml
96-317	10 ml
96-317B	32 ml

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

Guidelines for Vacuum Manifold

Recommended Settings

The following is required for use with the Vacuum/Spin Protocol:

A) **Vacuum Manifold**
Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector

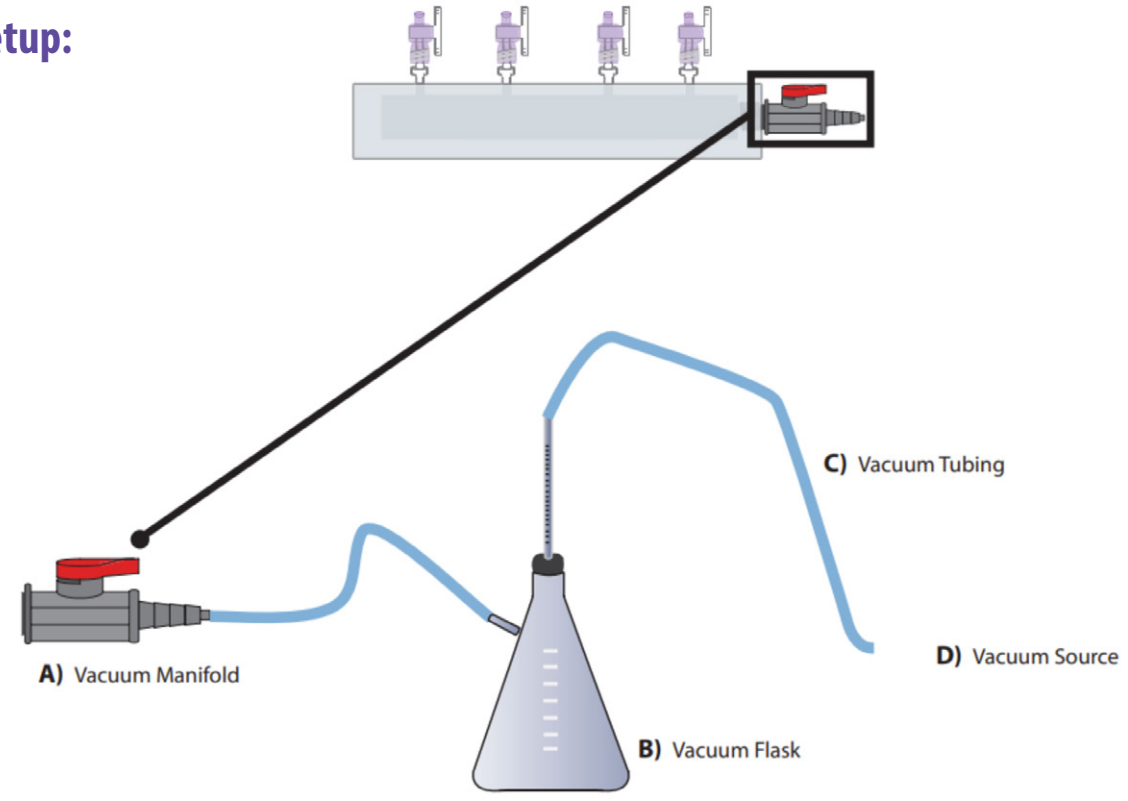
B) **Vacuum Flask**

C) **Vacuum Tubing**

D) **Vacuum Source** (review tables below for pressure settings)

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

Vacuum Setup:



Genesee Scientific A Life Science Company
CALL: 800.789.5550 • **FAX:** 888.789.0444 • **WEB:** www.geneseesci.com • **EMAIL:** support@geneseesci.com

Protocol for Tissue

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 LB1 Buffer and LB2 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 µl LB1 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
- 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 LB3 Buffer according to the directions in the "Preparing Reagents" section
- 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 LB1 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 LB1 Buffer used: for a 40-60 mg sample use 400 µl LB1 Buffer.

3. Add 25 µl Proteinase K 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 LB2 Buffer. Adjust the volume of LB2 Buffer based on the amount of starting material. Vortex to mix thoroughly

Example: If you used 400 µl of LB1 Buffer then add 420 µl LB2 Buffer and 420 µl 100% ethanol.

Note: A wispy precipitate may form upon the addition of LB2 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 DNA Mini Column into a 2 ml Collection Tube

11. Transfer the entire sample from **Step 9** to the 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 LB3 Buffer

Note: LB3 Buffer must be diluted with 100% isopropanol before use. Please see "Preparing Reagents."

15. Centrifuge at maximum speed for 1 minute

16. Discard the filtrate and reuse the collection tube

17. Insert the 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 100% ethanol before use. Please see "Preparing Reagents."

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 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 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

Protocol for 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
- 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 LB3 Buffer according to the directions in the "Preparing Reagents" section
- 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 Proteinase K 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 LB2 Buffer

Note: A wispy precipitate may form upon the addition of LB2 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 DNA Mini Column into a 2 ml Collection Tube

7. Transfer the entire sample from **Step 5** to the 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 LB3 Buffer

Note: LB3 Buffer must be diluted with 100% isopropanol before use. Please see "Preparing Reagents."

11. Centrifuge at maximum speed for 30 seconds

12. Discard the filtrate and collection tube

13. Insert the 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 100% ethanol before use. Please see "Preparing Reagents."

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 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 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

Protocol for 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
- 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 LB3 Buffer according to the directions in the "Preparing Reagents" section
- 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 LB1 Buffer

3. Add 25 µl Proteinase K 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.

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 LB2 Buffer and one volume 100% ethanol. Vortex to mix thoroughly
Example: If you transfer 180 µl cleared lysate, add 180 µl LB2 Buffer and 180 µl 100% ethanol.

Note: A wispy precipitate may form upon the addition of LB2 Buffer. This is does not interfere with DNA recovery.

8. Insert a DNA Mini Column into a 2 ml Collection Tube

9. Transfer the entire sample from **Step 7** to the 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 LB3 Buffer
Note: LB3 Buffer must be diluted with 100% isopropanol before use. Please see "Preparing Reagents."

13. Centrifuge at maximum speed for 30 seconds

14. Discard the filtrate and collection tube

15. Insert the 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 100% ethanol before use. Please see "Preparing Reagents."

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 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 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

Protocol for 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
- 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 LB3 Buffer according to the directions in the "Preparing Reagents" section
- 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 LB1 Buffer

11. Add 25 µl 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 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 LB2 Buffer. Adjust the volume of LB2 Buffer based on the amount of starting material. Vortex to mix thoroughly
Note: A wispy precipitate may form upon the addition of LB2 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 DNA Mini Column into a 2 ml Collection Tube

20. Transfer the entire sample from **Step 18** to the 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 LB3 Buffer
Note: LB3 Buffer must be diluted with 100% isopropanol before use. Please see "Preparing Reagents."

24. Centrifuge at maximum speed for 30 seconds

25. Discard the filtrate and collection tube

26. Insert the 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 100% ethanol before use. Please see "Preparing Reagents."

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 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 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 LB1 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

Protocol for 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
- 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 LB3 Buffer according to the directions in the "Preparing Reagents" section
- 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 Proteinase K Solution

3. Add 250 µl LB2 Buffer. Vortex to mix thoroughly

Note: A wispy precipitate may form upon the addition of LB2 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 DNA Mini Column into a 2 ml Collection Tube

7. Transfer the entire sample from **Step 5** to the 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 LB3 Buffer
Note: LB3 Buffer must be diluted with 100% isopropanol before use. Please see" Preparing Reagents.".

11. Centrifuge at maximum speed for 30 seconds

12. Discard the filtrate and collection tube

13. Insert the 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 100% ethanol before use. Please see "Preparing Reagents."

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 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 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

Notes

Notes

CALL: 800.789.5550 • **FAX:** 888.789.0444 • **WEB:** www.geneseesci.com • **EMAIL:** support@geneseesci.com

Notes

Vacuum Manifold/Spin Protocol

Carry out disruption, homogenization, protease digestion, and loading onto the 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 13,000 x *g*
- Nuclease-free 1.5 ml microcentrifuge tubes
- Shaking water bath, heat block, or incubator capable of 70°C
- Vortexer
- 100% ethanol
- 100% isopropanol

Before Starting:

- Set water bath, heat block, or incubator to 70°C
- Prepare DNA Wash Buffer and LB3 Buffer according to the directions in the "Preparing Reagents" section
- Heat Elution Buffer to 70°C

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

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

3. Transfer the entire sample to the 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 LB3 Buffer

Note: LB3 Buffer must be diluted with 100% isopropanol before use. Please see" Preparing Reagents."

7. Switch on vacuum source to draw the LB3 Buffer through the column

8. Turn off the vacuum

9. Add 700 µl DNA Wash Buffer
Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see "Preparing Reagents."

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 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**.

Possible Problems and Suggestions

Problem	Cause	Solution
Clogged Column	Incomplete lysis	• Extend lysis time with LB1 Buffer and Proteinase K Solution
	Sample size is too large	• If using more than 30 mg tissue, increase volumes of Proteinase K Solution, LB1 Buffer, LB2 Buffer, and ethanol
	Sample is viscous	• Divide sample into multiple tubes and adjust the volume to 250 µl with LB1 Buffer
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 • LB3 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, LB1 Buffer, LB2 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
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 LB2 Buffer	• Repeat the procedure, make sure to vortex the sample thoroughly with LB2 Buffer

Notes

GENE CHOICE®

A Genesee Scientific Brand