E.Z.N.A.® MicroElute® Genomic DNA Kit

Revision No: 2.0

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	D3096-00	D3096-01	D3096-02
Purifications	5	50	200
MicroElute® LE DNA Columns	5	50	200
2 mL Collection Tubes	10	100	400
BL Buffer	5 mL	35 mL	130 mL
TL Buffer	5 mL	35 mL	125 mL
HBC Buffer	5 mL	25 mL	80 mL
Proteinase K Solution	150 µL	1.2 mL	4.5 mL
LPA Buffer	25 µL	125 µL	500 µL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL
Elution Buffer	15 mL	30 mL	30 mL

Supplied by user:

- Microcentrifuge capable of at least 13,000 x *g*
- Water baths, heat blocks, or incubators capable of 55°C and 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol
- 100% isopropanol
- PBS if using Blood, Serum, or Body Fluids Protocol
- 3M NaOH
- Sterile deionized water
- Optional: RNase A (25 mg/mL)

Before starting:

- Prepare HBC Buffer and DNA Wash Buffer according to the instructions on the bottles.
- Set water baths, heat blocks, or incubators to 55°C and 70°C.
- Heat Elution Buffer to 70°C.

DNA Purification from Tissue Samples

LYSE

1. Mince up to 10 mg tissue and transfer to a 1.5 mL microcentrifuge tube (not provided).
2. Add 200 µL TL Buffer. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.
3. Incubate at 55°C in a shaking water bath. If a shaking water bath is not available, vortex the sample every 10-20 minutes. Lysis time will depend on the amount and type of tissue, but is usually less than 3 hours. Lysis can proceed overnight.
4. Centrifuge at maximum speed (≥13,000 x *g*) for 2 minutes.
5. Transfer the supernatant to a clean 1.5 mL microcentrifuge tube.

Optional: Certain tissues such as liver 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. Add 5 µL RNase A (25 mg/mL, assuming a sample size of 10 mg) and incubate at room temperature for 2 minutes. Proceed to Step 6.

BIND

6. Add 220 µL BL Buffer. Vortex to mix thoroughly.

   Note: If LPA Buffer is needed, add 1-2 µL LPA Buffer to 220 µL BL Buffer.
7. Incubate at 70°C for 10 minutes.
8. Add 220 µL 100% ethanol. Vortex at maximum speed for 15 seconds. A precipitate may form at this point; it will not interfere with DNA isolation. Briefly centrifuge to bring down any liquid from the top of the lid.
9. Insert a MicroElute® LE DNA Column into a 2 mL Collection Tube (provided) and follow the column equilibration steps listed below:

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BIND

Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® LE DNA Column.
2. Centrifuge at 10,000*g* for 30 seconds.
3. Add 500 µL sterile deionized water to the MicroElute® LE DNA Column.
4. Centrifuge at 10,000*g* for 30 seconds.
5. Discard the filtrate and reuse the collection tube.

10. Transfer the sample from Step 8 (including any precipitate that may have formed) to the MicroElute® LE DNA Column.
11. Centrifuge at maximum speed for 1 minute. Discard the filtrate and the collection tube.
12. Transfer the MicroElute® LE DNA Column to a new 2 mL Collection Tube.

WASH

13. Add 500 µL HBC Buffer diluted with 100% isopropanol (see the bottle for instructions). Centrifuge at maximum speed for 30 seconds. Discard the filtrate and reuse the collection tube.
14. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.
15. Repeat Step 14 for a second DNA Wash Buffer wash step.
16. Centrifuge the empty MicroElute® LE DNA Column for 2 minutes at maximum speed to dry the column matrix. It is important to dry the column matrix before elution. Residual ethanol may interfere with downstream applications.

ELUTE

17. Transfer the MicroElute® LE DNA Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube.
18. Add 10-50 µL Elution Buffer (or sterile deionized water) heated to 70°C. Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.
19. Let sit at room temperature for 3 minutes. Centrifuge at maximum speed for 1 minute. Store DNA at -20°C.

DNA Purification from Small Volumes of Blood, Serum, or Body Fluids

LYSE

1. Add 1-100 µL sample (the sample must be at room temperature) to a 1.5 mL microcentrifuge tube (not provided). Adjust the sample volume to 100 µL with PBS.
2. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.
3. Add 120 µL BL Buffer. Vortex to mix thoroughly.
4. Incubate at 70°C for 10 minutes.
5. Add 160 µL isopropanol. Vortex at maximum speed for 15 seconds. A precipitate may form at this point; it will not interfere with DNA isolation. Briefly centrifuge to bring down any liquid from the top of the lid.

BIND

6. IInsert a MicroElute® LE DNA Column into a 2 mL Collection Tube (provided) and follow the column equilibration steps listed below:

Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® LE DNA Column.
2. Centrifuge at 10,000*g* for 30 seconds.
3. Add 500 µL sterile deionized water to the MicroElute® LE DNA Column.
4. Centrifuge at 10,000*g* for 30 seconds.
5. Discard the filtrate and reuse the collection tube.

7. Transfer the sample from Step 5 (including any precipitate that may have formed) to the MicroElute® LE DNA Column.
8. Proceed to Step 11 of the DNA PURIFICATION FROM TISSUE SAMPLES protocol above.