E.Z.N.A.® Tissue DNA Kit MODIFIED Quick Guide

[Standard Quick Guide in drawer]

Product	D3396-02	Storage	Product	D3396-02	Storage
HiBind® DNA Mini Columns	200	Box [room temp]	HBC Buffer*	80 mL	Box
2 mL Collection Tubes	400	Box	DNA Wash Buffer*	3 x 25 mL	Box
BL Buffer	60 mL	Box	Elution Buffer	2 X 50 mL	Box
TL Buffer	60 mL	Box	OB Protease Solution	6.0 mL	Walk-in fridge [4°C]

*Record date alcohol added in notebook

DNA Extraction and Purification from 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 samples, but additional TL Buffer and BL Buffer will need to be purchased separately.

- ☐ 1. Set heat block to 55°C.
- ☐ 2. Mince up to 30 mg tissue and transfer to a *labeled* nuclease-free 1.5-mL microcentrifuge tube.
- ☐ 3. Add 200 µL TL Buffer.
  - For samples >30 mg, increase volume of TL Buffer; for a 40-60 mg sample, use 400 µL.
- ☐ 4. Add 25 µL OB Protease Solution.
- ☐ 5. Vortex to mix thoroughly, then briefly spin down.
- ☐ 6. Incubate at 55°C in shaking heat block (≥ 600 rpm) for minimum of 3 hours.
- ☐ 7. Centrifuge at maximum speed (≥10,000 x *g*) for 5 minutes, and set heat block to 70°C.
- ☐ 8. Transfer supernatant to a *labeled* nuclease-free 1.5-mL microcentrifuge tube. **Do not** disturb or transfer any of the insoluble pellet or remaining tissues.
- ☐ 9. Add 220 µL BL Buffer.
  - Adjust volume of BL Buffer based on the amount of starting material.
  - A wispy precipitate may form upon the addition of BL Buffer. This does not interfere with DNA recovery.
- ☐ 10. Vortex to mix thoroughly, then briefly spin down.
- ☐ 11. Incubate samples at 70°C for 10 minutes. Meanwhile, insert a HiBind® DNA Mini Column into a 2 mL Collection Tube and label columns accordingly.
- ☐ 12. Add 220 µL 100% ethanol to samples.
  - Adjust volume of ethanol based on the amount of starting material.
- ☐ 13. Vortex to mix thoroughly, the briefly spin down.
- ☐ 14. Transfer the entire sample to the HiBind® DNA Mini Column, including any precipitates that may have formed.
- ☐ 15. Centrifuge at maximum speed for 1 minute. Discard filtrate and **reuse** collection tube.
- ☐ 16. Add 500 µL HBC Buffer to the column, then centrifuge at maximum speed for 30 seconds.
- ☐ 17. Discard filtrate and collection tube. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.
- ☐ 18. Aliquot 200 µL* of Elution Buffer **per sample** into a nuclease-free 1.5-mL microcentrifuge tube [ex: 4 samples = heat 800 µL of Elution Buffer; *see Step 25].
- ☐ 19. Heat Elution Buffer aliquot(s) to 70°C on block.
- ☐ 20. Add 700 µL DNA Wash Buffer to the column, then centrifuge at maximum speed for 30 seconds.
- ☐ 21. Discard filtrate and **reuse** collection tube.
- ☐ 22. Repeat Steps 20-21 for a second DNA Wash Buffer wash step.
- ☐ 23. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column.
  - This step is critical for removal of trace ethanol that may interfere with downstream applications.
- ☐ 24. Transfer the HiBind® DNA Mini Column into a nuclease-free *labeled* 1.5-mL microcentrifuge tube.
- ☐ 25. Add 100 µL Elution Buffer heated to 70°C directly to filter of column.
- ☐ 26. Let sit at room temperature for 2 minutes, then centrifuge at maximum speed for 1 minute.
- ☐ 27. Repeat Steps 25-26 for a second elution step.
- ☐ 28. Check concentration of samples via Qubit, then store samples accordingly.
  - When necessary also check protein content via Nanodrop, and distribution of fragments on electrophoresis gel.
  - Store eluted DNA at 4°C if entering library preparation within 1-2 days, -20°C for long-term storage.