EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Appendix P_Omega E.Z.N.A. FFPE DNA

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP review complete

Standard Operating Procedure Approval Page:

Date Implemented:	23 January 2008	
Updated:	Ashima Amin **Name**	15 June 2020 **Date**
Supersedes:	Ashima Amin **Name**	11 April 2016 **Date**

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

Changes Made:
061520: Review, Protocols split out into appendixes. Renumbered SOPs. Previously EIGC.003.

Changes to previous procedures:
041116: Review, LIMS steps updated.

Note: No laboratory personnel should use this piece of equipment without proper training by the laboratory supervisor or other designated person.

Introduction:

E.Z.N.A.® FFPE DNA Kit combines MicroElute® LE DNA Column technology with a proprietary buffer system to provide a fast and easy method for DNA isolation from FFPE samples. There is no need for phenol/chloroform extraction and time-consuming steps, such as precipitation with isopropanol or ethanol, are eliminated. DNA purified using the E.Z.N.A.® FFPE DNA method is ready for applications such as PCR.

Kit Contents:

Product	**D3399-01**
Number of Purifications	50 preps
MicroElute® LE DNA Columns	50
2 mL Collection Tubes	150
BL Buffer	12 mL
FTL2 Buffer	12 mL
HBC Buffer[a]	25 mL
DNA Wash Buffer[b]	25 mL
Elution Buffer	30 mL
Proteinase K Solution[c]	1.5 mL

[a] Resuspend with 10 mL 100% isopropanol.

[b] Resuspend with 100 mL 100% ethanol.

[c] Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store Proteinase K Solution at 2-8°C.

Safety: All employees are required to follow universal safety protocols when working with potentially infectious biohazardous materials. Safety procedures are outlined in the Emory University Bloodborne Pathogen Exposure Control Plan (see Employee Safety Notebook).

Extraction Recording Keeping and LIMS: The extraction date is recorded in the LIMS system as the date DNA is received. Extraction kit lot numbers are recorded in the QC report that is passed on to the client.

Procedural Steps:

Notes before starting

- Heat the water bath or heat block to 55°C
- Heat the water bath or heat block to 90°C
- Heat Elution Buffer to 70°C for the elution step
- Prepare the HBC Buffer and DNA Wash Buffer according to the instructions in the table above. Date and initial both tubes once prepared.
- You will need the following additional equipment and reagents:
    - 100% ethanol
    - 100% isopropanol
    - Microcentrifuge set to room temperature
    - RNase A, 20 mg/mL

1. **Prepare the FFPE tissue for extraction:**
    a. **Scrape tissue from slides (preferably around 8 slides, cut at 5-10 µm thick). Some investigators need tissue from the entire slide, and some investigators will indicate which tissue to scrape by circling the tissue or by referencing which tissue to scrape on an H&E stained slide.**
    b. **Investigator may provide scrolls of paraffin embedded tissue (preferably from 3-8 slides, cut at 5-10 µm thick).**

2. **Immediately add 200 µL FTL2 Buffer to the tubes containing FFPE tissue.**

3. **Vortex for 20 s to mix thoroughly.**

4. **Incubate at 90°C for 15 min to melt the paraffin. Mix the sample a few times by gently shaking the tube 2-3 times. Make sure that the tissue sections stay submerged in the solution.**

5. **Let sit at room temperature for 5 min to allow to cool before adding Proteinase K Solution.**
   Note: If the sample temperature is too high, Proteinase K can be inactivated.

6. **Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.**

7. **Incubate at 55°C for 3 h.**
   Note: Incubation can proceed overnight.

8. **Centrifuge the tube briefly to collect any liquid adhering to the lid.**

9. **Add 10 µL RNase A (20 mg/mL, not provided) and incubate for 5 min at room temperature.**

10. **Add 220 µL BL Buffer. Vortex to mix thoroughly.**

11. **Add 250 µL 100% ethanol. Vortex to mix thoroughly.**

12. **Insert a MicroElute® LE DNA Column in a 2 mL Collection Tube.**

13. **Transfer the entire sample from Step 12 (including any precipitate that may have formed) to the MicroElute® LE DNA Column.**

14. **Centrifuge at 10,000 x g for 1 min at room temperature.**
    Note: If the filtrate does not go through the column, you can incubate the tube at 55°C for a few min and re-centrifuge. Heating the column will usually allow the filtrate to pass through the column.

15. **Discard the filtrate and the collection tube.**

16. **Transfer the MicroElute® LE DNA Column to a new 2 mL Collection Tube.**

17. **Add 500 µL HBC Buffer.**
    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see above for instructions.

18. **Centrifuge at 10,000 x g for 1 min at room temperature.**

19. **Discard the filtrate and the collection tube.**

20. **Transfer the MicroElute® LE DNA Column to a new 2 mL Collection Tube.**

21. **Add 700 µL DNA Wash Buffer.**
    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

22. **Centrifuge at 10,000 x g for 1 min at room temperature.**

23. **Discard the filtrate and reuse the collection tube.**

24. **Repeat Steps 21-23 for a s DNA Wash step.**

25. **Centrifuge the empty MicroElute® LE DNA Column at maximum speed for 2 min to dry the column.**

Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

26. **Transfer the MicroElute® LE DNA Column into a new 1.5 mL microcentrifuge tube.**

27. **Add 50 µL Elution Buffer heated to 70°C directly to the center of the column membrane.**

28. **Let sit for 3 min at room temperature.**

29. **Centrifuge at maximum speed for 1 min to elute DNA.**

30. **Repeat Steps 28-30 for a second elution step, (performance of this step depends on the starting amount of the sample).**