E.Z.N.A.® FFPE 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	D3399-00	D3399-01
Purifications	5	50
MicroElute® LE DNA Columns	5	50
2 mL Collection Tubes	15	150
BL Buffer	1.5 mL	12 mL
FTL2 Buffer	1.5 mL	12 mL
HBC Buffer	5 mL	25 mL
DNA Wash Buffer	2.5 mL	25 mL
Elution Buffer	2 mL	30 mL
Proteinase K Solution	150 µL	1.5 mL

Supplied by user:

- Tabletop microcentrifuge capable of 14,000*g*
- Water baths or heat blocks capable of 37°C, 55°C, 70°C, and 90°C
- Vortexer
- Nuclease-free 1.5 or 2 mL microcentrifuge tubes
- Nuclease-free pipette tips
- 100% ethanol
- 100% isopropanol
- Xylene
- 3M NaOH
- Sterile deionized water
- Optional: RNase A (20 mg/mL)

Before starting:

- Prepare HBC Buffer and DNA Wash Buffer according to the directions on the bottles.
- Set water baths or heat blocks to 37°C, 55°C, 70°C, and 90°C.
- Heat Elution Buffer to 70°C.

DNA Extraction and Purification from FFPE - Xylene Method

LYSE

1. Add 1 mL xylene to a 1.5 mL or 2 mL microcentrifuge tube.
2. Cut 3-8 paraffin sections 5-10 µm thick. Do not use the first 2-3 sections. Immediately place the section(s) into the xylene. Vortex for 20 seconds to mix thoroughly.
3. Centrifuge at maximum speed for 2 minutes. Aspirate and discard the supernatant. Do not disturb the pellet.
4. Add 1 mL 100% ethanol. Vortex to mix thoroughly.
5. Centrifuge at maximum speed for 2 minutes. Aspirate and discard the supernatant. Do not disturb the pellet.
6. With the lid open, dry the pellet at 37°C for 15 minutes. Carefully aspirate any residual ethanol with a pipettor before proceeding to the next step.
7. Add 200 µL FTL2 Buffer and pipet up and down to resuspend the pellet.
8. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.
9. Incubate at 55°C for 3 hours. Incubation can proceed overnight.
10. Incubate at 90°C for 10-30 minutes. Centrifuge the tube briefly to collect any liquid adhering to the lid.

Optional: If RNA-free gDNA is required, add 10 µL RNase A (20 mg/mL, not provided) and let sit for 5 minutes at room temperature.

BIND

11. Add 220 µL BL Buffer. Vortex to mix thoroughly.
12. Add 250 µL 100% ethanol. Vortex to mix thoroughly.
13. Insert a MicroElute® LE DNA Column into a 2 mL Collection Tube (provided) and follow the column equilibration steps listed below:

BIND

Protocol for Column Equilibration:

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

14. Transfer the entire sample from Step 12 (including any precipitates) to the MicroElute® LE DNA Column.
15. Centrifuge at 10,00 *g* for 1 minute. Discard the filtrate and the collection tube.

WASH

16. Transfer the MicroElute® LE DNA Column to a new 2 mL Collection Tube.
17. Add 500 µL HBC Buffer diluted with 100% isopropanol (see the bottle for instructions). Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and the collection tube.
18. Transfer the MicroElute® LE DNA Column to a new 2 mL Collection Tube.
19. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and reuse the collection tube.
20. Repeat Step 19 for a second DNA Wash step.
21. Centrifuge the empty MicroElute® LE DNA 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.

ELUTE

22. Place the MicroElute® LE DNA Column into a new 1.5 mL microcentrifuge tube.
23. Add 50-75 µL Elution Buffer heated to 70°C. Let sit for 3 minutes at room temperature. Centrifuge at maximum speed for 1 minute.
24. Repeat Step 23 for a second elution step.
25. Store eluted DNA at -20°C.

DNA Extraction and Purification - Heat Method

DIGEST & LYSE

1. Add 200 µL FTL2 Buffer into a 1.5 mL or 2 mL microcentrifuge tube (not provided).
2. Cut 3-4 paraffin sections 5-10 µm thick. Do not use the first 2-3 sections. Immediately place the section(s) into the FTL2 Buffer. Vortex for 20 seconds.
3. Incubate at 90°C for 15 minutes. Gently shake the tube 2-3 times. Make sure that the sections stay submerged.
4. Let sit at room temperature for 5 minutes to cool before adding Proteinase K Solution. If the sample temperature is too high, Proteinase K can be inactivated.
5. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.
6. Incubate at 55°C for 3 hours. Incubation can proceed overnight.
7. Centrifuge the tube briefly to collect any liquid adhering to the lid.

   Optional: If RNA-free gDNA is required, add 10 µL RNase A (20 mg/mL, not provided) and let sit for 5 minutes at room temperature.
8. Proceed to Step 11 of the DNA EXTRACTION AND PURIFICATION - XYLENE METHOD protocol on the reverse page.