omega BIO-TEK
innovations in nucleic acid isolation

# Product Manual

## E.Z.N.A.® FFPE DNA Kit

| | |
|---|---|
| D3399-00 | 5 preps |
| D3399-01 | 50 preps |

**Manual Date: September 2022**
**Revision Number: v8.0**

**For Research Use Only**


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# E.Z.N.A.® FFPE DNA Kit

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# Introduction and Overview

E.Z.N.A.® FFPE DNA Kit provides a rapid and easy method for the isolation of genomic DNA from FFPE tissue sections. 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.

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. The sample is heat-treated with FTL2 Buffer followed by Proteinase K digestion to release DNA. After adjusting the binding conditions with ethanol, the lysate is applied to the MicroElute® LE DNA Column to bind DNA. Cellular debris and proteins are effectively removed during the wash steps. High-quality DNA is eluted in sterile deionized water or low salt buffer.

**Binding Capacity:** Each MicroElute® LE DNA Column can bind approximately 100 µg DNA. Using greater than 30 mg FFPE tissue is not recommended.

**New in this Edition:**

September 2022
- Column equilibration protocol is now required for more consistent results.

January 2019
- Omega Bio-tek has a new logo.

February 2017
- FTL Buffer has been replaced with FTL2 Buffer.

December 2013
- The MicroElute® DNA Mini Columns have been replaced with MicroElute® LE DNA Columns. The redesign increases DNA recovery by reducing the HiBind® matrix retention volume.

# Kit Contents

| Product | D3399-00 | D3399-01 |
|---|---|---|
| Number of Purifications | 5 preps | 50 preps |
| 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 |
| User Manual | ✓ | ✓ |

## Storage and Stability

All components of the E.Z.N.A.® FFPE DNA Kit can be stored at room temperature and are guaranteed for at least 12 months from the date of purchase. 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. Under cool ambient conditions, a precipitate may form in the BL Buffer. In case of such an event, heat the bottle at 37°C to dissolve.

# Preparing Reagents

1. Dilute HBC Buffer with 100% isopropanol as follows and store at room temperature.

| Kit | 100% Isopropanol to be Added |
|---|---|
| **D3399-00** | 2 mL |
| **D3399-01** | 10 mL |

2. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

| Kit | 100% Ethanol to be Added |
|---|---|
| **D3399-00** | 10 mL |
| **D3399-01** | 100 mL |

## E.Z.N.A.® FFPE DNA Kit Protocol - Xylene Extraction Method

**Materials and Equipment to be Supplied by User:**

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

**Before Starting:**

- Heat the water bath or heat block to 37°C
- 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 Preparing Reagents section on Page 4

1. Add 1 mL xylene to a 1.5 mL or 2 mL microcentrifuge tube (not provided).

2. Cut 3-8 paraffin sections 5-10 µm thick.

   **Note:** Do not use the first 2-3 sections.

3. Immediately place the section(s) into the tube containing xylene.

4. Vortex for 20 seconds to mix thoroughly.

5. Centrifuge at maximum speed (≥13,000*g*) for 2 minutes at room temperature.

6. Aspirate and discard the supernatant. Do not disturb the pellet.

7. Add 1 mL 100% ethanol. Vortex to mix thoroughly.

8. Centrifuge at maximum speed for 2 minutes at room temperature.

9. Aspirate and discard the supernatant. Do not disturb the pellet.

10. 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.

11. Add 200 µL FTL2 Buffer and pipet up and down to resuspend the pellet.

12. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

13. Incubate at 55°C for 3 hours.

    **Note:** Incubation can proceed overnight.

14. Incubate at 90°C for 10-30 minutes.

15. 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.

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

17. Add 250 µL 100% ethanol. Vortex to mix thoroughly.

18. Insert 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.

19. Transfer the entire sample from Step 17 (including any precipitate that may have formed) to the MicroElute® LE DNA Column.

20. Centrifuge at 10,000*g* for 1 minute at room temperature.

21. Discard the filtrate and the collection tube.

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

23. Add 500 µL HBC Buffer.

    **Note:** HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 4 for instructions.

24. Centrifuge at 10,000*g* for 1 minute at room temperature.

25. Discard the filtrate and the collection tube.

26. Transfer the MicroElute® LE DNA Column to 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 Page 4 for instructions.

28. Centrifuge at 10,000*g* for 1 minute at room temperature.

29. Discard the filtrate and reuse the collection tube.

30. Repeat Steps 27-29 for a second DNA Wash step.

31. Centrifuge the empty MicroElute® LE DNA 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 MicroElute® LE DNA Column into a new 1.5 mL microcentrifuge tube.

33. Add 50-75 µL Elution Buffer heated to 70°C directly to the center of the column membrane.

34. Let sit for 3 minutes at room temperature.

35. Centrifuge at maximum speed for 1 minute.

36. Repeat Steps 33-35 for a second elution step.

37. Store eluted DNA at -20°C.

## E.Z.N.A.® FFPE DNA Kit Protocol - Heat Extraction Method

**Materials and Equipment to be Supplied by User:**

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

**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 Preparing Reagents section on Page 4

**Note:** All centrifugation steps must be performed at room temperature.

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.

   **Note:** Do not use the first 2-3 sections.

3. Immediately place the section(s) into the tube containing FTL2 Buffer.

4. Vortex for 20 seconds to mix thoroughly.

5. Incubate at 90°C for 15 minutes 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.

6. Let sit at room temperature for 5 minutes to allow to cool before adding Proteinase K Solution.

   **Note:** If the sample temperature is too high, Proteinase K can be inactivated.

7. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

8. Incubate at 55°C for 3 hours.

   **Note:** Incubation can proceed overnight.

9. 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 incubate for 5 minutes 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 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.

13. Transfer the entire sample from Step 11 (including any precipitate that may have formed) to the MicroElute® LE DNA Column.

14. Centrifuge at 10,000*g* for 1 minute at room temperature.

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 Page 4 for instructions.

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

23. Discard the filtrate and reuse the collection tube.

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

25. Centrifuge the empty MicroElute® LE DNA 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.

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

27. Add 50-75 µL Elution Buffer heated to 70°C directly to the center of the column membrane.

28. Let sit for 3 minutes at room temperature.

29. Centrifuge at maximum speed for 1 minute to elute DNA.

30. Repeat Steps 27-29 for a second elution step.

31. Store eluted DNA at -20°C.

## E.Z.N.A.® FFPE DNA Kit Protocol - Vacuum Method

**Materials and Equipment to be Supplied by User:**

- Vacuum manifold
- Vacuum source or pump

The vacuum method outlined here is an alternative to centrifugation steps presented in the protocols above. Either the xylene or heat extraction method can be used to remove the paraffin prior to DNA extraction via the vacuum method. Carry out deparaffinization, Proteinase K digestion, and column equilibration as indicated in either of the two preceding protocols. Instead of continuing with the initial sample transfer to the MicroElute® LE DNA Column (Step 19 in the Xylene Method, Page 7 or Step 13 in the Heat Method, Page 10), follow the steps below.

**Note:** Please read through the preceding protocols of this manual before using this protocol.

1. Prepare the vacuum manifold according to manufacturer's instructions. Connect the MicroElute® LE DNA Column to the manifold 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. Switch on vacuum source to draw the buffer through the column.
3. Turn off the vacuum.
4. Add 500 µL sterile deionized water to the MicroElute® LE DNA Column.
5. Switch on vacuum source to draw the water through the column.
6. Turn off the vacuum.

2. Load the sample (from Step 17 in the Xylene Method, Page 6 or Step 11 in the Heat Method, Page 10) onto MicroElute® LE DNA Column.

3. Turn on vacuum source to draw the sample through the column.

4. Turn off the vacuum.

5. Add 500 µL HBC Buffer.

   **Note:** HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 4 for instructions.

6. Turn on vacuum source to draw the HBC Buffer through the column.

7. Turn off the vacuum.

8. Add 700 µL DNA Wash Buffer.

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

9. Turn on vacuum source to draw the DNA Wash Buffer through the column.

10. Turn off the vacuum.

11. Repeat Steps 8-10 for a second DNA Wash step.

12. Remove the column from the vacuum manifold and transfer to a new 2 mL Collection Tube.

13. Centrifuge at full speed 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.

14. Place the MicroElute® LE DNA Column into a new 1.5 mL microcentrifuge tube.

15. Add 50-75 µL Elution Buffer heated to 70°C directly to the center of the column membrane.

16. Let sit for 3 minutes at room temperature.

17. Centrifuge at maximum speed for 1 minute to elute DNA.

18. Repeat Steps 15-17 for a second elution step.

19. 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, toll free, at **(800-832-8896)**.

| Problem | Cause | Solution |
|---|---|---|
| Low or No DNA eluted | Incomplete cell lysis or crosslinking removal | Increase incubation time with FTL2 Buffer and protease. Ensure that no visible pieces of tissue remain. |
| | Samples are rich in protein | After applying to column, wash with 300 µL of a 1:1 mixture of BL Buffer and ethanol and then with DNA Wash Buffer. |
| | Poor cell lysis due to improper mixing with BL Buffer | Mix thoroughly with BL Buffer prior to loading the MicroElute® LE DNA Column. |
| | Poor cell and/or protein lysis in FTL2 Buffer | Tissue sample must be cut or minced into small pieces. Increase incubation time at 55°C with FTL2 Buffer to ensure that tissue is completely lysed. |
| | Ethanol not added to DNA Wash Buffer | Dilute DNA Wash Buffer with the indicated volume of 100% ethanol before use. |
| | Isopropanol not added to HBC Buffer | Dilute HBC Wash Buffer with the indicated volume of 100% isopropanol before use. |
| | Column Equilibration not performed | Perform the column equilibration protocol as instructed in the manual for consistent results. |
| **Problem** | **Cause** | **Solution** |
| Washing leaves colored residue in column | Incomplete lysis due to improper mixing with BL Buffer | BL Buffer is viscous and the sample must be vortexed thoroughly. |
| | Ethanol not added to DNA Wash Buffer | Dilute DNA Wash Buffer with the indicated volume of 100% ethanol before use. |

For more purification solutions, visit **www.omegabiotek.com**

## AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

## SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

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