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

June 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® FFPE DNA Kit

Table of Contents

Manual Revision: June 2013

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Introduction

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.

Overview

E.Z.N.A.® FFPE DNA Kit combines MicroElute® DNA Mini 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 FTL Buffer followed by Proteinase K digestion to release DNA. After adjusting the binding conditions with ethanol, the lysate is applied to the MicroElute® DNA Mini Column to bind DNA. Cellular debris and proteins are effectively washed away during the wash steps. High-quality DNA is eluted in sterile deionized water or low salt buffer.

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

New in this Edition:

- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D3399-00	D3399-01
Number of Purifications	5 preps	50 preps
MicroElute® DNA Mini Columns	5	50
2 mL Collection Tubes	15	150
BL Buffer	1.5 mL	12 mL
FTL Buffer	1.5 mL	12 mL
HBC Buffer	4 mL	25 mL
DNA Wash Buffer	1.5 mL	15 mL
Elution Buffer	1 mL	10 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. Store BL Buffer at room temperature.

Preparing Reagents

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

Kit	100% Ethanol to be Added
D3399-00	6 mL
D3399-01	60 mL

2. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D3399-00	1.6 mL
D3399-01	10 mL

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

Materials and Equipment to be Supplied by User:

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

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 DNA Wash Buffer and HBC 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 for 2 minutes at room temperature.

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

7. Add 1 mL 100% ethanol to the tube and 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 FTL Buffer and pipette up and down to resuspend the pellet.

12. Add 20 µL Proteinase K Solution and 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 incubate 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. Place a MicroElute® DNA Mini Column in a 2 mL Collection Tube provided with this kit.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® DNA Mini Column.
2. Centrifuge at maximum speed for 30-60 seconds.
3. 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® DNA Mini Column.

20. Centrifuge at 10,000 x *g* for 1 minute at room temperature to bind the DNA to the column membrane.

21. Discard the filtrate and the collection tube.

22. Transfer the MicroElute® DNA Mini Column to a new 2 mL Collection Tube provided with this kit.

23. Add 500 µL HBC Buffer to the MicroElute® DNA Mini Column.

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

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

25. Discard the filtrate and the collection tube.

26. Transfer the MicroElute® DNA Mini Column to a new 2 mL Collection Tube provided with this kit.

27. Add 700 µL DNA Wash Buffer to the MicroElute® DNA Mini Column.

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

28. Centrifuge at 10,000 x *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 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.

32. Place the MicroElute® DNA Mini Column into a new 1.5 mL microcentrifuge tube (not provided).

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 to elute DNA.

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:

- 100% Ethanol
- Isopropanol
- Centrifuge capable of 14,000 x *g*
- 1.5 mL or 2 mL nuclease-free microcentrifuge tubes
- Nuclease-free pipette tips
- Water baths or heat blocks capable of 55°C, 70°C, and 90°C
- Optional: RNase A, 20 mg/mL
- Optional: 3M NaOH

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 DNA Wash Buffer and HBC 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 FTL 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 FTL 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. Leave sample 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 and 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 and vortex to mix thoroughly.

11. Add 250 µL 100% ethanol and vortex to mix thoroughly.

12. Place a MicroElute® DNA Mini Column in a 2 mL Collection Tube provided with this kit.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® DNA Mini Column.
2. Centrifuge at maximum speed for 30-60 seconds.
3. 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® DNA Mini Column.

14. Centrifuge at 10,000 x *g* for 1 minute at room temperature to bind the DNA to the column membrane.

15. Discard the filtrate and the collection tube.

16. Transfer the MicroElute® DNA Mini Column to a new 2 mL Collection Tube provided with this kit.

17. Add 500 µL HBC Buffer to the MicroElute® DNA Mini Column.

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

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

19. Discard the filtrate and the collection tube.

20. Transfer the MicroElute® DNA Mini Column to a new 2 mL Collection Tube provided with this kit.

21. Add 700 µL DNA Wash Buffer to the MicroElute® DNA Mini Column.

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

22. Centrifuge at 10,000 x *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 at full speed for 3 minutes to completely dry the membrane.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

26. Place the MicroElute® DNA Mini Column into a new 1.5 mL microcentrifuge tube (not provided).

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 (Cat# VAC-08)
- 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® DNA Mini Column (Step 22 in the Xylene Method, Page 7 or Step 16 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.

2. Connect the MicroElute® DNA Mini Column to the vacuum manifold.

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

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

5. Turn off the vacuum.

6. Add 500 µL HBC Buffer to the MicroElute® DNA Mini Column.

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

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

8. Turn off the vacuum.

9. Add 700 µL DNA Wash Buffer to the MicroElute® DNA Mini Column.

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

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

11. Turn off the vacuum.

12. Repeat Steps 9-11 for a second DNA Wash step.

13. Remove the column from the vacuum manifold and transfer to a new 2 mL collection tube provided with the kit.

14. 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.

15. Place the MicroElute® DNA Mini Column into a new 1.5 mL microcentrifuge tube (not provided).

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

17. Let sit for 3 minutes at room temperature.

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

19. Repeat Steps 16-18 for a second elution step.

20. 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
	Incomplete cell lysis or crosslinking removal	Increase incubation time with FTL 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.
Problem	**Cause**	**Solution**
No DNA eluted	Poor cell lysis due to improper mixing with BL Buffer	Mix thoroughly with BL Buffer prior to loading the MicroElute® DNA Mini Column.
	Poor cell and/or protein lysis in FTL Buffer	Tissue sample must be cut or minced into small pieces. Increase incubation time at 55°C with FTL 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 isopropanol before use.
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.

Notes: