## Contents


Introduction . . . . . 2

Principle . . . . . 2

Storage and Stability . . . . . 2

Binding Capacity . . . . . 2

Kit Contents . . . . . 3

Before Starting . . . . . 3

E.Z.N.A.™ Standard Protocol for FFPE Tissues . . . . . 4

E.Z.N.A.™ Alternative Protocol for FFPE Tissues . . . . . 6

E.Z.N.A.™ Vacuum/Spin Protocol for FFPE Tissues . . . . . 9

Troubleshooting Guide . . . . . 10



**Revised June 2009**


## 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 extractions, 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*.

## Principle

E.Z.N.A.™ FFPE DNA Kit combined the HiBind spin column technology with a proprietary buffer system to provide a fast and easy method for DNA isolation from FFPE samples. The sample is first heat-treated with FTL Buffer followed by proteinase K digestion to release DNA. After adjust the binding condition with ethanol,the lysate is applied to the HiBind® spin columns to bind DNA, cellular debris, hemoglobin, and other proteins are effectively washed away. High quality DNA is finally eluted in sterile deionized water or low salt buffer.

## Storage and Stability

All components of the E.Z.N.A.™ FFPE DNA Kit, except the Protease K can be stored at 22°C-25°C and are guaranteed for at least 12 months from the dated of purchase. Once reconstituted in water, Protease K must be stored at -20°C. Under cool ambient conditions, a precipitate may form in the Buffer BL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer BL at room temperature.

## Binding Capacity

Each HiBind®column can bind approximately 100 µg DNA. Using greater than 30 mg FFPE tissue is not recommended.

---

*The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 (and international equivalents) owned by Hoffmann-LaRoche, Inc.

## Kit Contents

| Product Number | D3399-00 | D3399-01 | D3399-02 |
|---|---|---|---|
| Purification Times | 5 Preps | 50 Preps | 200 Preps |
| MicroElute DNA Columns | 5 | 50 | 200 |
| 2 ml Collection Tubes | 15 | 150 | 600 |
| Buffer BL | 5 ml | 20 ml | 60 ml |
| Buffer FTL | 5 ml | 20 ml | 60 ml |
| Equilibration Buffer | 1.5 mL | 7 mL | 25 mL |
| Buffer HB | 3 ml | 30 ml | 120 ml |
| DNA Wash Buffer | 1.5 ml | 15 ml | 2 x 25 ml |
| Elution Buffer | 2 ml | 20 ml | 60 ml |
| Proteinase K | 3 mg | 30 mg | 4 x 30 mg |
| Proteinase Storage Buffer | 160 µl | 3 ml | 12 ml |
| User Manual | 1 | 1 | 1 |

## Preparing Reagents

<table>
<tr><td rowspan="2">IMPORTANT</td><td>1. Reconstitute Proteinase K in 150 µl (5 Preps) or 1.5 ml (50 and 200 preps) Protease Storage Buffer in each tube. Vortex vial briefly prior to use. We recommend that you aliquot and store vials of reconstituted protease at -20°C.</td></tr>
<tr><td>2 DNA Wash Buffer must be diluted with absolute (~96-100%)ethanol as follows:<br>D3399-00 Add 6 ml absolute ethanol<br>D3399-01 Add 60 ml absolute ethanol/ bottle<br>D3399-02 Add 100 ml absolute ethanol/ bottle</td></tr>
</table>

## E.Z.N.A.™ FFPE DNA Isolation Protocol

**Materials and Equipments supplied by user**

- Table-top microcentrifuge capable of 13,000 x g
- Nuclease-free pipette tips
- Water bath or heat block Capable of 90°C
- Water bath or heat block Capable of 55°C
- Water bath or heat block Capable of 70°C
- Absolute Ethanol (~96-100%)
- Rnase A 20 mg/mL (optional)

### Before Starting:

- Prepare Reagents according to Page 3
- Set a water Bath or Heat Block to 90°C
- Set a water Bath or Heat Block to 55°C

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

1. Using a scalpel, trim excess paraffin off the sample block. **Cut sections 10-20um thick.** If the sample surface has been exposed to air, discard the first 2-3 sections.

2. Immediately transfer 3-8 sections in a 1.5 ml tube and add 200 µl Buffer FTL. **Vortex to mix. Incubate at 90°C for 15 minutes.**

3. Sit at room temperature for 5 minutes to allow the sample to cool to room temperature before adding Protease.

4. **Add 20 µl Proteinase K and mix by vortexing for 15 seconds. Then Incubate at 55°C for 1-3 hours or overnight.**

5. Briefly centrifuge the tube to collect any drops from the inside of the lid. If RNA-Free genomic DNA if required, add 10 µl RNase(20mg/ml) and incubate for 5 min at room temperature.

6. **Add 230 µl Buffer BL and vortex to mix throughly by vortexing for 20**

**seconds.**

7. **Add 250 µl absolute ethanol and mix thoroughly by vortexing for 30 seconds.**

8. Add 100 µL of Equilibration Buffer to the MicroElute DNA column in a 2 mL Collection Tube (Provided). Centrifuge at 10,000 x g for 30 **seconds.** Discard flow-through and reuse the collection tube.

9. **Transfer the entire sample from step 7 into the column including any precipitate that may have formed.** Centrifuge at 10,000 x g for 1 min to bind DNA. Discard the collection tube and flow-through liquid.

10. **Place the column into a new2 ml collection tube(provided) and wash by pipetting 500 ul of Buffer HB to the MicroElute DNA Column.** Centrifuge at 10,000 x g for 1 min. Discard collection tube and flow-through liquid.

11. **Place the column into a new 2 ml collection tube (provided) and wash by pipetting 700 µl of DNA Wash Buffer diluted with ethanol to the MicroElute DNA column.** Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse the collection tube.

12. **Using the same 2 ml collection tube , wash the column with a second 700 µl of DNA Wash Buffer**. Centrifuge at 10,000 x g for 1 min. Discard the flow-through and re-use the collection tube.

13. Place the empty column into the same collection tune from previous step and centrifuge at maximum speed (> 13,000 x g) for 3 minutes to dry the column membrane.

    **Note: Completely dry the column before elution step is critical for DNA quality. If the column is not completely dried, the ethanol residue from DNA wash Buffer could interfere the down stream applications.**

14. **Place the column into a sterile 1.5 ml microcentrifuge tube and add 50-75 µl of preheated (70°C) Elution Buffer**. Allow tubes to sit for 3 min at room temperature.

15. **To elute DNA from the column, centrifuge at maximum speed (>13,000 x g ) for 1 min.** Repeat the elution with a second 50-75 µl of Elution Buffer.

**Alterative Protocol For DNA Isolation from FFPE**

Note: the following protocol is designed for FFPE samples with low DNA content. This protocol

**Materials and Equipments supplied by user**

- 96-100% ethanol
- Xylene
- Table-top microcentrifuge
- Nuclease-free pipette tips
- Water bath or heat block Capable of 90°C
- Water bath or heat block Capable of 55°C
- Water bath or heat block Capable of 70°C
- Water bath or heat block Capable of 37 °C

**Before Starting:**

- Prepare Reagents according to Page 3
- Heat elution Buffer to 70°c for Elution Step
- Set a water Bath or Heat Block to 90°C
- Set a water Bath or Heat Block to 55°C

1. Using a sclpel, trim excess paraffin off the sample block. **Cut the FFPE Sample into small pieces or cut sections 10-20 um thick.** If the sample surface has been exposed to air, discard the first 2-3 sections.

2. **Immediately transfer 3-8 sections or <20 mg samples in a 1.5 ml tube. Add 1 ml xylene and mix thoroughly by vortexing for 10s.**

3. **Centrifuge the tube at 10,000 x g for 2 min. Discard supernatant without disturbing the tissue pellet.**

4. **Rinse the pellet with 1 ml absolute ethanol to remove traces of xylene.** Centrifuge at maximum speed (>13,000 x g) for 2 min. Discard

the ethanol without disturbing the tissue pellet. Carefully remove any residual ethanol using a fine pipet tip.

5. Open the tube and air dry tissue pellet at 37°C for 15 min or until all residual ethanol has evaporated.

6. **Add 200 µl Buffer FTL and 20 µl Proteinase K and mix by vortexing**. Then incubate at 55°C for 3 hours or overnight.

7. **Incubate at 90°C for 10-30 minutes.**

8. Centrifuge the tube briefly to collect any drops from the inside of the lid. If RNA-Free genomic DNA if required, add 10 µl Rnase(20mg/ml) and incubate for 5 min at room temperature.

9. **Add 220 µl Buffer BL and vortex to mix.**

10. **Add 250 µl absolute ethanol and mix thoroughly by vortexing.**

11. Add 100 µL of Equilibration Buffer to the MicroElute DNA column in a 2 mL Collection Tube (Provided). Centrifuge at 10,000 x g for 30 **seconds.** Discard flow-through and reuse the collection tube.

12. **Transfer the entire sample from step 10 into the column including any precipitate that may have formed**. Centrifuge at 10,000 x g for 1 min to bind DNA. Discard the collection tube and flow-through liquid.

13. Place the column into a new 2 ml collection tube(provided) and wash by pipetting 500 ul of Buffer HB to the Micro elute DNA Column. Centrifuge at 10,000 x g for 1 min. Discard collection tube and flow-through liquid.

14. **Place the column into a new 2 ml collection tube and wash by pipetting 700 µl of DNA Wash Buffer diluted with ethanol.** Centrifuge at 10,000 x g for 1 min. Discard flow-through and re-use the collection tube.

15. **Using the same 2 ml collection tube , wash the column with a second 700 µl of DNA Wash Buffer**. Centrifuge at 10,000 x g for 1 min. Discard the flow-through and re-use the collection tube.

16. Place the empty column into the same collection tune from previous step and centrifuge 13,000 x g for 3 minutes to dry the column membrane.

    **Note: Completely dry the column before elution step is critical for DNA quality. If the column is not completely dried, the ethanol residue from DNA wash Buffer could interfere the down stream applications.**

17. **Place the column into a sterile 1.5 ml microcentrifuge tube and add 50-75 µl of preheated (70°C) Elution Buffer**. Allow tubes to sit for 3 min at room temperature

18. **To elute DNA from the column, centrifuge at maximum speed (>13,000 x g ) for 1 min**. Repeat the elution with a second 50-75 µl of Elution Buffer**.**

# Vacuum/Spin Protocol

Carry out disruption, homogenization, Protease digestion, and loading onto HiBind® DNA column as indicated previous protocols. Instead of continuing with centrifugation, follow steps blow.

Note: Please read through previous section of this book before using this protocol.

1. Prepare the vacuum manifold according to manufacturer' s instruction and connect the HiBind® DNA V-Spin column to the manifold.

2. Load the sample into HiBind® DNA V-spin column.

3. Switch on vacuum source to draw the sample through the column and turn off the vacuum.

4. Wash the column by adding 500 μl Buffer HB, draw the buffer through the column by turn on the vacuum source.

5. Wash the column by adding 500 μl DNA wash buffer, draw the wash buffer through the column by turn on the vacuum source.

6. Wash the column again by adding 500 μl DNA wash buffer, draw the wash buffer through the column by turn on the vacuum source.

7. Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin 2 minute to dry the column.

8. Place the column in a clean 1.5 ml micro-centrifuge tube and add 20-100 μl Elution Buffer. Stand for 1-2 minute and centrifuge 1 minute to elute DNA.

## Troubleshooting Guide

| Problem | Possible Cause | Suggestions |
|---|---|---|
| Clogged Column | Incomplete lysis | Extend incubation time of lysis with Buffer FTL and protease. It may be necessary to extend incubation time by 48hours. |
| | Sample too large | If using more than 30 mg tissue, increase volumes of Proteinase K, Buffer FTL, Buffer BL, and ethanol. Pass aliquots of lysate through one column successively. |
| Low DNA yield | Clogged column | See above |
| | Poor elution | Repeat elution or increase elution volume (see note on page 4). Incubation of column at 70°C for 5 min with Elution Buffer may increase yields. |
| | Improper washing | Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on page 4 before use. |
| Low $A_{260}/A_{280}$ ratio | Extended centrifugation during elution step. | Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation — it will not interfere with PCR or restriction digests. |
| | Poor cell lysis due to incomplete mixing with Buffer BL | Repeat the procedure, this time making sure to vortex the sample with Buffer BL immediately and completely. |

| Problem | Possible Cause | Suggestions |
|---|---|---|
| | Incomplete cell lysis or protein degradation due to insufficient incubation. | Increase incubation time with Buffer FTl 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 Buffer BL and ethanol and then with DNA Wash Buffer. |
| No DNA eluted | Poor cell lysis due to improper mixing with Buffer BL. | Mix thoroughly with Buffer BL prior to loading HiBind® column. |
| | Poor cell and/or protein lysis in Buffer TL. | Tissue sample must be cut or minced into small pieces. Increase incubation time at 55°C with Buffer FTL to ensure that tissue is completely lysed. |
| | Absolute ethanol not added to Buffer BL. | Before applying sample to column, an aliquot of Buffer BL/ethanol must be added. See protocol above. |
| | No ethanol added to Wash Buffer Concentrate. | Dilute Wash Buffer with the indicated volume of absolute ethanol before use. |
| Washing leaves colored residue in column | Incomplete lysis due to improper mixing with Buffer BL. | Buffer BL is viscous and the sample must be vortexed thoroughly. |
| | No ethanol added to Wash Buffer Concentrate. | Dilute Wash Buffer with the indicated volume of absolute ethanol before use. |

## Order Information

| Product | Applications | Cat. No. |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |