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Revised March 2008

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 uses the reversible binding properties of HiBind® matrix, a new silica-based material, combined with the speed of mini-column spin technology. A specifically formulated buffer system allows genomic DNA up to 60 kb to bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® spin columns to which DNA binds, while 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 OB Protease can be stored at 22°C-25°C and are guaranteed for at least 24 months from the dated of purchase. Once reconstituted in water, OB Protease 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 50 µg DNA. Using greater than 30 mg FFPE tissue is not recommended.

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*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
HiBind® DNA MicroElute Columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer BL	5 ml	20 ml	60 ml
Buffer TL	5 ml	20 ml	60 ml
Buffer HB	5 ml	30 ml	120 ml
DNA Wash Buffer	2 ml	20 ml	2 x 25 ml
Protease Storage Buffer	200 ul	1.8 ml	7 ml
Elution Buffer	2 ml	20 ml	60 ml
OB Protease	3 mg	30 mg	4 x 30 mg
User Manual	1	1	1

Before Starting

<table>
<tr><td rowspan="2">IMPORTANT</td><td>1. Reconstitute OB Protease 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 -20ºC.</td></tr>
<tr><td>2 DNA Wash Buffer must be diluted with absolute (~96-100%)ethanol as follows:
D3399-00 Add 8 ml absolute ethanol
D3399-01 Add 80 ml absolute ethanol / bottle
D3399-02 Add 100 ml absolute ethanol / bottle</td></tr>
</table>

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

E.Z.N.A.™ Standard Protocol For FFPE Tissue

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 10,000 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 37ºC for 15 min or until all residual ethanol has evaporated.

6. **Add 200 µl Buffer TL and 20 µl OB Protease and mix by vortexing. Then incubate at 55ºC for 3 hours or overnight.**

7. **Incubate at 90ºC for 60 minutes.**

8. Briefly centrifuge the tube to collect any drops from the inside of the lid. If RNA-Free genomic DNA if required, add 2 µl Rnase(100mg/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. Assemble an HiBind® DNA MicroElute column in a 2 ml collection tube (provided). **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.

12. **Place the column into a second 2 ml collection tube and wash by pipetting 500 ul of Buffer HB.** Centrifuge at 10,000 x g for 1 min. Again, dispose of collection tube and flow-through liquid.

13. **Place the column into a new 2 ml collection tube and wash by pipetting 500 µl of DNA Wash Buffer diluted with ethanol.** Centrifuge at 10,000 x g for 1 min. Discard flow-through.
    Note: DNA Wash Buffer Concentrate must be diluted with absolute ethanol before use. See label for directions.

14. **Using the same 2 ml collection tube , wash the column with a second 500 µl of DNA Wash Buffer**. Centrifuge at maximum speed (10,000 x g) for 3 min to dry the column. **This step is crucial for ensuring optimal elution in the following step**.

15. **Place the column into a sterile 1.5 ml microcentrifuge tube and add 20-100 µl of preheated (70ºC) Elution Buffer**. Allow tubes to sit for 3 min at room temperature.

16. **To elute DNA from the column, centrifuge at 10,000 x g for 1 min**. Repeat the elution with a second 20-100 µl of Elution Buffer.

E.Z.N.A.™ Fast Protocol For FFPE Tissue

1. Using a sclpel, 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 TL. **Vortex to mix. Incubate at 90ºC for 30 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 OB Protease and mix by vortexing. Then Incubate at 55ºC for 1-3 hours or overnight.**

5. Proceed step 8-16 of standard protocol on page 4-5.

E.Z.N.A.™ Vacuum/Spin Protocol For FFPE Tissue

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 TL 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 OB Protease or Proteinase K, Buffer TL, 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 70ºC 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.
	Incomplete cell lysis or protein degradation due to insufficient incubation.	Increase incubation time with Buffer Tland protease. Ensure that no visible pieces of tissue remain.

Problem	Possible Cause	Suggestions
	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 55ºC with Buffer TL 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.