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Introduction

E.Z.N.A.™ FFPE RNA Kit provides a rapid and easy method for the isolation of Total RNA 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. RNA purified using the E.Z.N.A.™ FFPE RNA method is ready for applications such as RT-PCR, Northern Blot, etc*.

Principle

E.Z.N.A.™ FFPE RNA 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 RNA less than 20 bp to bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® spin columns to which RNA binds, while cellular debris, hemoglobin, and other proteins are effectively washed away. High quality RNA is finally eluted in sterile deionized water or low salt buffer.

Storage and Stability

All components of the E.Z.N.A.™ FFPE RNA 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 GTC. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer GTC at room temperature.

Binding Capacity

Each HiBind®column can bind approximately 50 µg RNA. 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	R6954-00	R6954-01	R6954-02
Purification Times	5 Preps	50 Preps	200 Preps
HiBind® RNA MicroElute Columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer GTC	5 ml	20 ml	60 ml
Buffer FTL	5 ml	20 ml	60 ml
RWB Wash Buffer	2 ml	12 ml	4 x 12 ml
DEPC-Treated Water	2 ml	10 ml	20 ml
Protease Storage Buffer	200 ul	1.8 ml	7 ml
OB Protease	3 mg	30 mg	4 x 30 mg
User Manual	1	1	1

Before Starting

IMPORTANT	1.	Reconstitute OB Protease in 120 µl (5 Preps) or 1.2 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.
	2	RWB Wash Buffer must be diluted with absolute (~96-100%)ethanol as follows: **R6954-00** Add 8 ml absolute ethanol **R6954-01** Add 48 ml absolute ethanol / bottle **R6954-02** Add 48 ml absolute ethanol / bottle

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

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

1. Using a scalpel, trim excess paraffin off the sample block. **Cut sections 5-10um 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 or 2 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 at room temperature. 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 at room temperature. 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 FTL and 20 µl OB Protease to the tissue, mix by vortexing.**

7. **Incubate at 55ºC for 15 min, then incubate at 80ºC for 15 min.**

8. **Centrifuge at 10,000xg for 5 min. Transfer the supernatant into a new tube.**

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

10. **Add 660 µl absolute ethanol and mix thoroughly by vortexing.**

11. **Assemble an HiBind® RNA MicroElute column in a 2 ml collection tube (provided).** Transfer 700ul of the 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 RNA. Discard flow-through liquid and reuse the collection tube in step 12.

12. Repeat step 11 until the entire sample has passed through the HiBind RNA MicroElute Column. Discard flow-through liquid and the collection tube.

13. **Place the column into a new 2 ml collection tube and wash by pipetting 500 µl of RWB Wash Buffer diluted with ethanol.** Centrifuge at 10,000 x g for 1 min. Discard flow-through.

Note: RWB Wash Buffer Concentrate must be diluted with absolute ethanol before use. See label for directions. If refrigerated, RWB Wash Buffer must be brought to room temperature before use.

14. **Using the same 2 ml collection tube , wash the column with a second 500 µl of RWB Wash Buffer**. Centrifuge at maximum speed (10,000 x g) for 2 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 15-50 µl of DEPC Tteated Water**. Allow tubes to sit for 3 min at room temperature.

16. **To elute RNA from the column, centrifuge at 10,000 x g for 1 min**.

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

1. Using a scalpel, trim excess paraffin off the sample block. **Cut sections 5-10um 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 or 2 ml tube. **Add 250 µl Buffer FTL to the tissue, mix by vortexing.**

3. Incubate at 80°C for 15 min. Incubate for 1 minutes at room temperature.

4. Immediately add 20 µl OB Protease to the tissue, mix by vortexing.

4. Incubate at 55°C for 15-60 min.

5. Centrifuge at 10,000 x g at room temperature for 3 minutes. The paraffin will form a thin layer on top of the lysate solution.

6. Use a 1 ml pipette tip or large orifice tip to penetrate the paraffin layer, transfer 200 µl of lysate into a new 1.5 ml tube.

7. Following step 9-16 of Standard protocol on page 4-5.

E.Z.N.A.™ Vacuum/Spin Protocol (V-Spin column only)

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® RNA V-Spin column to the manifold.

2. Load the sample into HiBind® RNA 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 650 µl RWB Wash Buffer, draw the buffer through the column by turn on the vacuum source.

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

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

7. Place the column in a clean 1.5 ml micro-centrifuge tube and add 15-50 µl DEPC-Treated Water. Stand for 1-2 minute and centrifuge 1 minute to elute RNA.

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 OB Protease or Proteinase K, Buffer FTL, Buffer GTC, and ethanol. Pass aliquots of lysate through one column successively.
Low RNA yield	Clogged column	See above
	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 FTL	Repeat the procedure, this time making sure to vortex the sample with Buffer FTL 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.
	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.

Problem	Possible Cause	Suggestions
No RNA eluted	Poor cell lysis due to improper mixing with Buffer GTC.	Mix thoroughly with Buffer GTC prior to loading HiBind® column.
	Poor cell and/or protein lysis in Buffer FTL.	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.	Before applying sample to column, Add 1.5 volume of absolute ethanol. See protocol above.
	No ethanol added to Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.