EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Appendix G_Omega E.Z.N.A. FFPE RNA

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP review complete

Standard Operating Procedure Approval Page:

Date Implemented: 23 January 2008

Updated:	Ashima Amin **Name**	15 June 2020 **Date**
Supersedes:	Ashima Amin **Name**	11 April 2016 **Date**

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

Changes Made:
061520: Review, Protocols split out into appendixes. Renumbered SOPs. Previously EIGC.003.

Changes to previous procedures:
041116: Review, LIMS steps updated

Note: No laboratory personnel should use this piece of equipment without proper training by the laboratory supervisor or other designated person.

Introduction: E.Z.N.A.® FFPE RNA Kit provides a rapid and easy method for the isolation of total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. Nucleic acids in FFPE samples are heavily fragmented and often modified by formaldehyde due to fixation and embedding procedures. The specially formulated buffers in E.Z.N.A.® FFPE RNA Kit are designed to minimize the effects of the formaldehyde modification and partially reverse cross-linking without the need for overnight digestion resulting in high-yielding, high-quality nucleic acids. Purified RNA is suitable for a variety of downstream applications including qRT-PCR, reverse transcription PCR, primer extension, expression array assays, microarray analyses, and next generation sequencing.
The E.Z.N.A.® FFPE RNA Kit combines the reversible binding properties of HiBind® RNA technology with a specially designed buffer system to quickly and efficiently isolate RNA from FFPE samples. This system includes a DNA Clearance Column that selectively binds and eliminates DNA contamination from the sample prior to RNA isolation. Briefly, an FFPE sample is de-paraffinized by either heat or xylene and digested with Proteinase K to release nucleic acids. The lysate is passed through a DNA Clearance Column that selectively binds the genomic DNA. The filtrate from the DNA Clearance Column is mixed with ethanol to optimize RNA binding conditions before loading the sample onto a MicroElute® LE RNA Column. With a brief centrifugation, the sample passes through the column where the RNA binds to the HiBind® matrix. After two rapid wash steps, purified RNA is eluted with Nuclease-free Water.

Kit Contents:

Catalog no.	R6954-00	R6954-01
Preparations	5	50
MicroElute® LE RNA Columns	5	50
DNA Clearance Columns	5	50
2 mL Collection Tubes	10	100
GPL Buffer	1 ml	10 ml
GFC Buffer	2 ml	20 ml
RNA Wash Buffer II [a]	2.5 ml	12 ml
Proteinase K Solution	110 µl	1.1 ml
Nuclease-free Water	2 ml	2 ml

[a] RNA Wash Buffer II is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (100%) as indicated on the bottle to obtain a working solution

All E.Z.N.A.® FFPE RNA Kit components are guaranteed for at least 12 months from the date of receiving when stored at room temperature. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage, store at 2-8°C. GFC Buffer is light sensitive, keep protected from light when not in use. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Safety: All employees are required to follow universal safety protocols when working with potentially infectious biohazardous materials. Safety procedures are outlined in the Emory University Bloodborne Pathogen Exposure Control Plan (see Employee Safety Notebook).

Extraction Recording Keeping and LIMS: The extraction date is recorded in the LIMS system as the date DNA is received. Extraction kit lot numbers are recorded in the QC report that is passed on to the client.

Procedural Steps:

Notes before starting

- Heat the water bath or heat block to 55°C.
- Heat the water bath or heat block to 80°C.
- Prepare the RNA Wash Buffer according to the instructions in the table above. Date and initial both tubes once prepared.
- You will need the following additional equipment and reagents:
    - 100% Ethanol
    - Microcentrifuge set to room temperature

1. **Prepare the FFPE tissue for extraction:**
    a. **Scrape tissue from slides (preferably around 8 slides, cut at 5-10 µm thick). Some investigators need tissue from the entire slide, and some investigators will indicate which tissue to scrape by circling the tissue or by referencing which tissue to scrape on an H&E stained slide.**
    b. **Investigator may provide scrolls of paraffin embedded tissue (preferably from 3-8 slides, cut at 5-10 µm thick).**

2. **Immediately add 140 µl GPL Buffer to the tubes containing FFPE tissue.**

3. **Vortex for 20 s to mix thoroughly.**

4. **Briefly centrifuge to collect the sample in the solution. Check the lids of the tubes for possible residues of paraffin.**

5. **Incubate at 80°C for 15 min 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. **Add 20 µl Proteinase K Solution. Vortex to mix thoroughly.**

7. **Incubate at 55°C for 30 min.**

8. **Incubate at 80°C for 15 min.**

9. **Immediately centrifuge at 13,000 × g for 5 min. The paraffin will form a thin layer on top of the lysate solution.**

10. **Use a 1 ml pipette tip or large orifice pipette tip to penetrate the paraffin layer. Transfer 150 µl cleared lysate to a new 1.5 ml or 2 ml micro-centrifuge tube (not provided).**

11. **Add 300 µl GFC Buffer. Vortex to mix thoroughly.**

12. **Insert a DNA Clearance Column in a 2 ml Collection Tube provided with the kit.**

13. **Transfer sample to the DNA Clearance Column.**

14. **Centrifuge at 13,000 × g for 1 min. SAVE the filtrate and use for RNA isolation in the next step.**

15. **Add 675 µl 100% ethanol to the filtrate. Pipet up and down 20 times to mix thoroughly. (Do not centrifuge).**

16. **Insert a MicroElute® LE RNA Column in a 2 ml Collection Tube provided with this kit.**

17. **Transfer 700 µl of the sample (including any precipitate that may have formed) to the MicroElute® LE RNA Column.**

18. **Centrifuge at 13,000 × g for 30 s at room temperature.**

19. **Discard the filtrate and reuse the collection tube.**

20. **Repeat Steps 17-19 until the remaining sample from Step 18 has been transferred to the MicroElute® LE RNA Column.**

21. **Add 500 µl RNA Wash Buffer II to the MicroElute® LE RNA Column. Note: RNA Wash Buffer II must be diluted with 100% ethanol before use.**

22. **Centrifuge at 13,000 × g for 30 s at room temperature.**

23. **Discard the filtrate and reuse the collection tube.**

24. **Repeat Steps 21-23 for a second RNA Wash Buffer II wash step.**

25. **Centrifuge the empty column at full speed for 2 min 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® LE RNA Column into a new 1.5 ml microcentrifuge tube (not provided).**

27. **Add 15-30 µl Nuclease-free Water directly to the center of the column membrane. Volume will depend upon starting material (for plentiful tissue use 30 µl; for minimal tissue use 15 µl).**

28. **Centrifuge at maximum speed for 1 min to elute RNA.**

29. **Store eluted RNA at -80°C.**

Troubleshooting:

Problem	Cause	Solution
Little or no RNA eluted	RNA remains on column	• Repeat elution. • Heat Nuclease-free Water to 70°C prior to elution. • Incubate 5 minutes with water prior to elution.
	Column is overloaded	• Reduce the quantity of starting material.
Clogged column	Incomplete lysis	• Reduce the amount of starting material.
Degraded RNA	Source	• Follow protocol closely and work quickly. • Samples fragmented/modified during fixation.
	RNase contamination	• Ensure not to introduce RNases during the procedure. • Check buffers for RNase contamination.
Problem with downstream applications	Salt carry-over during elution	• Ensure RNA Wash Buffer II has been diluted with 4 volumes of 100% ethanol as instructed on in Kit Contents Table • RNA Wash Buffer II must be stored at room temperature. • Repeat wash with RNA Wash Buffer II.
	Inhibitors of PCR	• Use less starting material.
Residual DNA contamination		• Digest with RNase-free DNase I and inactivate at 75°C for 5 minutes.