omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® FFPE RNA Kit

R6954-00	5 preps
R6954-01	50 preps

Manual Date: June 2023
Revision Number: v7.0

For Research Use Only

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek

E.Z.N.A.® FFPE RNA Kit

Table of Contents

Manual Date: June 2023
Revision Number: v7.0

omega BIO-TEK
innovations in nucleic acid isolation

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 deparaffinized 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 or vacuum step, 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.

New in this Edition:

June 2023
- GPL Buffer has been replaced by FTL2 Buffer. The functional performance of the kit is not affected by this buffer change.

September 2022
- Added Column Equilibration protocol for more consistent results.

March 2020:
- Storage and Stability section has been updated with change in GFC Buffer storage conditions.

November 2018:
- DEPC Water has been replaced with Nuclease-free Water. DEPC Water is no longer provided in this kit.
- PR032 (DEPC Water, 100 mL) has been discontinued and is no longer available to purchase.
- R6954-02 has been discontinued and is no longer available to purchase.

Kit Contents

Product	R6954-00	R6954-01
Preparations	5	50
MicroElute® LE RNA Columns	5	50
DNA Clearance Columns	5	50
2 mL Collection Tubes	10	100
FTL2 Buffer	1.5 mL	12 mL
GFC Buffer	2 mL	20 mL
RNA Wash Buffer II	2.5 mL	12 mL
Proteinase K Solution	110 µL	1.1 mL
Nuclease-free Water	2 mL	2 mL
User Manual	✓	✓

Storage and Stability

All E.Z.N.A.® FFPE RNA Kit components are guaranteed for at least 12 months from the date of purchase 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.

Preparing Reagents

Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol To Be Added
R6954-00	10 mL
R6954-01	48 mL

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips with the supplied reagents.
- Work quickly, but carefully.
- Under cool ambient conditions, crystals may form in some buffers. This is normal and the bottle should be warmed at 37°C to dissolve the precipitant.
- All centrifugation steps must be carried out at room temperature.

Note: Equilibrate samples and GFC Buffer to room temperature before beginning this protocol. All steps must be carried out at room temperature. Work quickly, but carefully.

Starting Materials

Since standard formalin fixation and paraffin-embedding procedures cause significant fragmentation of nucleic acids, we recommend the following guidelines to limit the extent of RNA fragmentation: 1) Use 4-10% formalin to fix tissue samples; 2) Limit the fixation time to 14-24 hours; and 3) Completely dehydrate samples before embedding. Always use freshly cut sections of FFPE tissue for RNA isolation. Although the binding capacity for each MicroElute® LE RNA Column is around 50 µg, the maximum amount of starting material depends on the type of the tissue being processed and its corresponding RNA content. It is essential to begin with the correct amount of tissue to get optimal RNA yield and purity with MicroElute® LE RNA Column. For the first time user, we recommend using 3-4 sections of 10 µm thickness. Depending on the yield and purity obtained, it may be possible to increase the starting material.

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

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000*g*
- Water baths or heat blocks capable of 55°C and 80°C
- 1.5 mL or 2 mL RNase-free microcentrifuge tubes
- RNase-free filter pipette tips
- 100% ethanol
- Xylene
- 3M NaOH
- Sterile deionized water

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 II 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 3-4 paraffin sample 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 3 minutes at room temperature.

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

7. Add 1 mL 100% ethanol to the tube. 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. Repeat Steps 7-9 for a second ethanol wash step.

11. Invert the tube on absorbent paper and air dry the pellet for 10 minutes at room temperature. Carefully remove any residual ethanol with a pipettor before proceeding to the next step.

12. Resuspend the pellet in 140 µL FTL2 Buffer.

13. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

14. Incubate for 15-30 minutes at 55°C.

15. Incubate for 15 minutes at 80°C.

16. Add 300 µL GFC Buffer. Vortex to mix thoroughly.

17. Insert a DNA Clearance Column in a 2 mL Collection Tube provided with this kit.

18. Add sample to the DNA Clearance Column.

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

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

21. Insert a MicroElute® LE RNA Column into a 2 mL Collection Tube (provided) and follow the column equilibration steps listed below:

Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® LE RNA Column.
2. Centrifuge at 10,000*g* for 30 seconds.
3. Add 500 µL sterile deionized water to the MicroElute® LE RNA Column.
4. Centrifuge at 10,000*g* for 30 seconds.
5. Discard the filtrate and reuse the collection tube.

22. Transfer 700 µL sample (including any precipitate that may have formed) to the MicroElute® LE RNA Column.

23. Centrifuge at 13,000*g* for 30 seconds at room temperature.

24. Discard the filtrate and reuse the collection tube.

25. Repeat Steps 22-24 until the remaining sample from Step 22 has been transferred to the MicroElute® LE RNA Column.

26. 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. Please see Page 4 for instructions.

27. Centrifuge at 13,000*g* for 30 seconds at room temperature.

28. Discard the filtrate and reuse the collection tube.

29. Repeat Steps 26-28 for a second RNA Wash Buffer II wash step.

30. Centrifuge the empty column 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.

31. Place the MicroElute® LE RNA Column into a new 1.5 mL microcentrifuge tube (not provided).

32. Add 15-30 µL Nuclease-free Water directly to the center of the column membrane.

33. Centrifuge at maximum speed for 1 minute to elute RNA.

34. Store eluted RNA at -70°C.

E.Z.N.A.® FFPE RNA Kit Protocol - Heat Method

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 13,000*g*
- Water baths or heat blocks capable of 55°C and 80°C
- 1.5 mL or 2 mL RNase-free microcentrifuge tubes
- RNase-free filter pipette tips
- 100% ethanol
- 3M NaOH
- Sterile deionized water

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 II according to the instructions in the Preparing Reagents section on Page 4

1. Add 140 µL FTL2 Buffer into a 1.5 mL or 2 mL microcentrifuge tube (not provided).

2. Cut 3-4 paraffin sample sections 5-10 µm thick.

   Note: Do not use the first 2-3 sections.

3. Immediately place the section(s) into the tube containing FTL2 Buffer.

4. Vortex for 20 seconds to mix thoroughly.

5. Briefly centrifuge to collect the sample in the solution.

6. Incubate for 15 minutes at 80°C 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.

7. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

8. Incubate for 15-30 minutes at 55°C.

9. Incubate for 15 minutes at 80°C.

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

11. Transfer 150 µL cleared lysate to a new 1.5 mL or 2 mL microcentrifuge tube (not provided).

    Note: Use a 1 mL pipette tip or large orifice pipette tip to penetrate the paraffin layer.

12. Add 300 µL GFC Buffer. Vortex to mix thoroughly.

13. Insert a DNA Clearance Column in a 2 mL Collection Tube provided with this kit.

14. Add sample to the DNA Clearance Column.

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

16. Add 675 µL 100% ethanol to the filtrate. Pipet up and down 20 times to mix thoroughly. **Do not centrifuge.**

17. Insert a MicroElute® LE RNA Column into a 2 mL Collection Tube (provided) and follow the column equilibration steps listed below:

Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® LE RNA Column.
2. Centrifuge at 10,000*g* for 30 seconds.
3. Add 500 µL sterile deionized water to the MicroElute® LE RNA Column.
4. Centrifuge at 10,000*g* for 30 seconds.
5. Discard the filtrate and reuse the collection tube.

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

19. Centrifuge at 13,000*g* for 30 seconds at room temperature.

20. Discard the filtrate and reuse the collection tube.

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

22. 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. Please see Page 4 for instructions.

23. Centrifuge at 13,000*g* for 30 seconds at room temperature.

24. Discard the filtrate and reuse the collection tube.

25. Repeat Steps 22-24 for a second RNA Wash Buffer II wash step.

26. Centrifuge the empty column 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.

27. Place the MicroElute® LE RNA Column into a new 1.5 mL microcentrifuge tube (not provided).

28. Add 15-30 µL Nuclease-free Water directly to the center of the column membrane.

29. Centrifuge at maximum speed for 1 minute to elute RNA.

30. Store eluted RNA at -70°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
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.
	Column Equilibration not performed	• Perform the column equilibration protocol as instructed in the manual for consistent results.
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 Page 4. • 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.

Notes:

Notes:

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

innovations in nucleic acid isolation

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek