omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® MicroElute® Total RNA Kit

R6831-00	5 preps
R6831-01	50 preps
R6831-02	200 preps

Manual Date: December 2022
Revision Number: v3.2

For Research Use Only

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E.Z.N.A.® MicroElute® Total RNA Kit

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Introduction and Overview

The E.Z.N.A.® MicroElute® Total RNA Kitprovides a rapid and easy method for the isolation of up to 50 µg total RNA from small amount of cultured eukaryotic cells and tissues such as laser dissected samples (LDS) or fine needle aspirates (FNA). Normally, up to 5 x 10^5 eukaryotic cells or 5 mg tissue (amounts depend on tissue type) can be used in a single experiment. The kit allows single or multiple, simultaneous processing of samples in less than 30 minutes. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or LiCl, are eliminated.

RNA purified using the E.Z.N.A.® MicroElute® Total RNA Kit is ready for applications such as RT-PCR*, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation.

The E.Z.N.A.® MicroElute® Total RNA Kit combines the reversible binding properties of the HiBind® matrix, a silica-based material, the speed of spin column technology , and a specially formulated high salt buffer system to allow RNA molecules greater than 200 bases to be purified. Cells or tissues are lysed under denaturing conditions that inactivate RNase. Once ethanol is added, samples are transferred to the MicroElute® LE RNA Columns to which total RNA binds, while cellular debris and other contaminants are effectively washed away. High-quality RNA is eluted in Nuclease-free Water.

New in this Edition:

December 2022
- General revision.

September 2022
- Added column equilibration protocol for more consistent results.

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.

Kit Contents

Product	R6831-00	R6831-01	R6831-02
Purifications	5	50	200
MicroElute® LE RNA Columns	5	50	200
2 mL Collection Tubes	10	100	400
TRK Lysis Buffer	5 mL	25 mL	100 mL
RWF Wash Buffer	5 mL	50 mL	200 mL
RNA Wash Buffer II	5 mL	12 mL	50 mL
Nuclease-free Water	15 mL	30 mL	60 mL
Carrier RNA	1 mg	1 mg	1 mg
User Manual	✓	✓	✓

Storage and Stability

All E.Z.N.A.® MicroElute® Total RNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Carrier RNA should be stored at -20°C. All other components should be stored at room temperature. During shipment, crystals or precipitates may form in TRK Lysis Buffer. Dissolve by warming buffer to 37°C with gentle shaking.

Quantification and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm with a spectrophotometer. One OD unit measured at 260 nm corresponds to 40 µg/mL RNA. Nuclease-free Water is slightly acidic and can lower A_{260}/A_{280} ratios. Use TE buffer to dilute RNA prior to spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while an A_{260}/A_{280} ratio of 0.6 denotes pure protein. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Phenol has a maximum absorbance at 270 nm and can interfere with spectrophotometric analysis of DNA or RNA. Store RNA samples at -70°C in water. Under these conditions, RNA is stable for more than a year.

Integrity of RNA

It is highly recommended that RNA quality be determined prior to beginning all downstream applications. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis with ethidium bromide staining. The ribosomal RNA bands should appear as sharp, clear bands on the gel. The 28S band should appear to be double that of the 18S RNA band (23S and 16S if using bacteria). If the ribosomal RNA bands in any given lane are not sharp and appear to be smeared towards the smaller sized RNA, it is very likely that the RNA undergone degradation during the isolation, handling, or storage procedure. Although RNA molecules less than 200 bases in length do not efficiently bind to the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

DNA Contamination

Generally HiBind® RNA spin column technology will efficiently remove most of the DNA without DNase treatment. However, no RNA extraction procedure can completely remove genomic DNA. For sensitive work (such as RT-PCR or differential display) we suggest that you perform on-column DNase I digestion (OBI cat# E1091) or treat the eluted RNA with RNase-free DNase. Also for RT-PCR, use intron-spanning primers that allow easy identification of DNA-contamination.

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 when using the supplied reagents.

- Equilibrate samples and reagents to room temperature before beginning this protocol. All steps should be carried out at room temperature unless otherwise noted. Work quickly, but carefully.

- Prepare all materials required before starting the procedure to minimize RNA degradation.

- Carefully apply the sample or solution to the center of the MicroElute® LE RNA Columns. Avoid touching the membrane with pipet tips.

- 2-mercaptoethanol is key in denaturing RNases and must be added to an aliquot of TRK Lysis Buffer before use. Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

Homogenization Techniques

Efficient sample disruption and homogenization is essential for successful total RNA isolation. Cell wall and plasma membrane disruption is necessary for the release of RNA from the sample and homogenization is necessary to reduce the viscosity of the lysates. Homogenization shears genomic DNA and other high-molecular-weight cell components creating a homogeneous lysate. Incomplete homogenization can cause the MicroElute® LE RNA Column to clog resulting in low or no yield.

Liquid Nitrogen Method

1. Wear appropriate gloves and take great care when working with liquid nitrogen.

2. Excise tissue and promptly freeze in a small volume of liquid nitrogen.

3. Grind tissue with a ceramic mortar and pestle under approximately 10 mL liquid nitrogen.

4. Pour the suspension into a pre-cooled 15 mL polypropylene tube.

   Note: Unless the tube is pre-cooled in liquid nitrogen, the suspension will boil vigorously and may cause loss of tissue.

5. Allow the liquid nitrogen to completely evaporate and add TRK Lysis Buffer.

6. Proceed to one of the homogenization steps below.

Homogenization - Choose one method below

1. Homogenizer Mini Columns (HCR003) and 2 mL Collection Tubes (AC-1370-00)

   - Load the lysate into a homogenizer spin column preinserted into a 2 mL Collection Tube (not provided).

   - Spin for two minutes at maximum speed in a microcentrifuge in order to collect homogenized lysate.

   - Proceed to Step 1 of the desired protocol below.

2. Syringe and Needle

   - Shear high-molecular-weight DNA by passing the lysate through a narrow needle (19-21 gauge) 5-10 times.

   - Proceed to Step 1 of the desired protocol below.

Rotor-Stator Homogenizer: Sample Disruption and Homogenization

Using a rotor-stator homogenizer for sample disruption and homogenization can simultaneously disrupt and homogenize most samples. The process usually takes less than a minute depending on sample type. Many rotor-stator homogenizers operate with differently sized probes or generators that allow sample processing in 50 mL tubes.

Bead Milling: Sample Disruption and Homogenization

By using bead milling, cells and tissue can be disrupted and homogenized by rapid agitation in the presence of glass beads and a lysis buffer. The optimal size of glass beads to use for RNA isolation are 0.5 mm for yeast/unicellular cells and 4-8 mm for animal tissue samples.

Syringe Needle: Sample Disruption and Homogenization

High-molecular-weight DNA is responsible for the viscosity of cell lysates and can be shredded by passing the sample 10-20 times through a narrow gauge needle (19-21 G).

Preparing Reagents

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

Kit	100% Ethanol to be Added
R6831-00	20 mL
R6831-01	48 mL
R6831-02	200 mL

2. Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

3. Optional: Prepare Carrier RNA Stock Solution. Dissolve Carrier RNA in Nuclease-free Water as follows and store in aliquots at -70°C.

Kit		Starting Material	Add	Concentration
R6831-00 R6831-01 R6831-02	Step 1	Carrier RNA	1 mL Nuclease-free Water	1 µg/µL
	Step 2	5 µL Carrier RNA (1 µg/µL)	45 µL TRK Lysis Buffer	100 ng/µL
	Step 3	5 µL Carrier RNA (100 ng/µL)	95 µL TRK Lysis Buffer	5 ng/µL

Protocol for Laser Dissected Samples

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000*g*
- Vortexer
- Nuclease-free pipette tips
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- 2-mercaptoethanol (14.3 M)
- 100% ethanol
- 70% ethanol
- 3M NaOH
- Sterile deionized water
- Optional: RNase-free DNase I Digestion Kit (Cat# E1091)

Before Starting:

- Prepare RNA Wash Buffer II and TRK Lysis Buffer according to the "Preparing Reagents" section on Page 8.
- Optional: Prepare a stock solution of Carrier RNA according to the "Preparing Reagents" section on Page 8.

1. Add 300 µL TRK Lysis Buffer to a 1.5 mL or 2 mL microcentrifuge tube (not provided).

   Note: Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer before use. Please see Page 8 for instructions.

   Optional: If using <5,000 cells, add 4 µL (20 ng) Carrier RNA Stock Solution before homogenization.

2. Transfer homogenized sample to the microcentrifuge tube containing TRK Lysis Buffer.

   Note: Refer to Pages 6-7 for information regarding different homogenization techniques.

3. Adjust the volume to 350 µL with TRK Lysis Buffer.

4. Vortex for 30 seconds to mix thoroughly.

5. Add 1 volume 70% ethanol. Vortex to mix thoroughly. Do not centrifuge.

   Note: A precipitate may form at this point. This will not interfere with the RNA purification.

6. Insert a MicroElute® LE RNA Column into 2 mL Collection Tube 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.

7. Transfer the entire lysate from Step 5, including any precipitates that may have formed, from Step 5 to the MicroElute® LE RNA Column.

8. Centrifuge at maximum speed (≥13,000*g*) for 15 seconds at room temperature.

9. Discard the filtrate and the Collection Tube.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the MicroElute® LE RNA Column eliminates most DNA, DNase I digestion is not necessary for most downstream applications. However, certain sensitive RNA applications may require further DNA removal. If an additional DNA removal step is required, please continue to the DNase I Digestion Protocol found on Page 22. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 10.

10. Transfer the MicroElute® LE RNA Column to a new 2 mL Collection Tube.

11. Add 500 µL RWF Wash Buffer.

12. Centrifuge at maximum speed for 30 seconds.

13. Discard the filtrate and reuse the collection tube.

14. Add 500 µL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 8 for instructions.

15. Centrifuge at maximum speed for 30 seconds.

16. Discard the filtrate and reuse the collection tube.

17. Repeat Steps 14-16 for a second RNA Wash Buffer II wash step.

18. Centrifuge at maximum speed for 2 minutes to completely dry the MicroElute® LE RNA Column.

    Note: It is important to dry the MicroElute® LE RNA Column matrix before elution. Residual ethanol may interfere with downstream applications.

19. Transfer the MicroElute® LE RNA Column to a clean 1.5 mL microcentrifuge tube.

20. Add 15-20 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the MicroElute® LE RNA Column matrix.

21. Centrifuge at maximum speed for 1 minute.

22. Store eluted RNA at -70°C.

Protocol for Microdissected Formalin-Fixed Tissues

Depending on the process of the fixation protocol, storage condition, staining protocol, and the age of the sample, RNA can be highly degraded into small fragments less than 300 nt. Since the Total RNA isolation protocol will remove most fragments less than 200 nt, this could lead to overall loss of RNA if the sample is highly degraded.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000*g*
- Vortexer
- Water bath or heat block capable of 55°C
- Nuclease-free pipette tips
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes 100% ethanol
- 2-mercaptoethanol (14.3 M)
- Proteinase K (20 mg/mL)
- 3M NaOH
- Sterile deionized water
- Optional: RNase-free DNase I Digestion Kit (Cat# E1091)

Before Starting:

- Prepare RNA Wash Buffer II and TRK Lysis Buffer according to the "Preparing Reagents" section on Page 8.
- Heat water bath or heat block to 55°C
- Optional: Prepare a stock solution of Carrier RNA according to the "Preparing Reagents" section on Page 8.

1. Add 100 µL TRK Lysis Buffer to a 1.5 mL or 2 mL microcentrifuge tube (not provided).

   Note: Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer before use. Please see Page 8 for instructions.

   Optional: If using <5,000 cells, add 4 µL (20 ng) Carrier RNA Stock Solution before homogenization.

2. Transfer homogenized sample to the microcentrifuge tube containing TRK Lysis Buffer.

   Note: Refer to Pages 6-7 for information regarding different homogenization techniques.

3. Adjust the volume to 150 µL with TRK Lysis Buffer.

4. Add 295 µL Nuclease-free Water.

5. Add 5 µL Proteinase K (20 mg/mL, not provided).

6. Incubate at 55°C for 10 minutes.

7. Centrifuge at maximum speed (≥13,000*g*) for 5 minutes at room temperature. A small pellet of tissue debris will form and a thin layer or film can be seen on top of the supernatant.

8. Transfer the supernatant (~450 µL) to a new nuclease-free 1.5 mL or 2 mL microcentrifuge tube.

   Note: Avoid transferring any of the pellet. Hold the pipette tip under the thin layer or film on top of the supernatant, if present. This layer will usually adhere to outside the tip and should not be transferred.

9. Add 0.5 volumes 100% ethanol. Vortex to mix thoroughly.

10. Insert a MicroElute® LE RNA Column into 2 mL Collection Tube 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.

11. Transfer the entire lysate, including any precipitates that may have formed, from Step 9 to the MicroElute® LE RNA Column.

12. Centrifuge at maximum speed for 15 seconds at room temperature.

13. Discard the filtrate and the collection tube.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the MicroElute® LE RNA Column eliminates most DNA, DNase I digestion is not necessary for most downstream applications. However, certain sensitive RNA applications may require further DNA removal. If an additional DNA removal step is required, please continue to the DNase I Digestion Protocol found on Page 22. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 14.

14. Transfer the MicroElute® LE RNA Column to a new 2 mL Collection Tube.

15. Add 500 µL RWF Wash Buffer.

16. Centrifuge at maximum speed for 30 seconds.

17. Discard the filtrate and reuse the collection tube.

18. Add 500 µL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 8 for instructions.

19. Centrifuge at maximum speed for 30 seconds.

20. Discard the filtrate and reuse the collection tube.

21. Repeat Steps 18-20 for a second RNA Wash Buffer II wash step.

22. Centrifuge at maximum speed for 2 minutes to completely dry the MicroElute® LE RNA Column.

    Note: It is important to dry the MicroElute® LE RNA Column matrix before elution. Residual ethanol may interfere with downstream applications.

23. Transfer the MicroElute® LE RNA Column to a clean 1.5 mL microcentrifuge tube.

24. Add 15-20 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the MicroElute® LE RNA Column matrix.

25. Centrifuge at maximum speed for 1 minute.

26. Store eluted RNA at -70°C.

Protocol for Animal Tissue or Cell Culture

This method is designed for most animal tissues and culture cells. For RNA isolation from fibrous tissue, follow the specialized protocol on Page 19. For laser dissected samples, please follow the protocol on Page 9. All centrifugation steps must be carried out at room temperature.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000*g*
- Vortexer
- Nuclease-free pipette tips
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes 100% ethanol
- 70% ethanol
- 2-mercaptoethanol (14.3 M)
- 3M NaOH
- Sterile deionized water
- Optional: RNase-free DNase I Digestion Kit (Cat# E1091)

Before Starting:

- Prepare RNA Wash Buffer II and TRK Lysis Buffer according to the "Preparing Reagents" section on Page 8.
- Optional:Prepare a stock solution of Carrier RNA according to the "Preparing Reagents" section on Page 8.

1. Determine the amount of starting material and homogenize the samples. Do not use more than 5 mg tissue or 5 x 10^5 cells.

   Note: Refer to Pages 6-7 for information regarding different homogenization techniques.

   Optional: If using <5,000 cells, add 4 µL (20 ng) Carrier RNA Stock Solution before homogenization.

2. Add 350 µL TRK Lysis Buffer to a 1.5 mL or 2 mL microcentrifuge tube (not provided).

   Note: Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer before use. Please see Page 8 for instructions.

3. Transfer homogenized sample to the microcentrifuge tube containing TRK Lysis Buffer.

4. Vortex for 30 seconds to mix thoroughly.

5. Centrifuge at maximum speed (≥13,000*g*) for 2 minutes.

6. Transfer the cleared supernatant to a new microcentrifuge tube.

7. Add 1 volume 70% ethanol. Vortex to mix thoroughly. Do not centrifuge.

   Note: A precipitate may form at this point. This will not interfere with the RNA purification.

8. Insert a MicroElute® LE RNA Column into 2 mL Collection Tube 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.

9. Transfer the entire lysate, including any precipitates that may have formed, from Step 7 to the MicroElute® LE RNA Column.

10. Centrifuge at maximum speed for 15 seconds at room temperature.

11. Discard the filtrate and the collection tube.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the MicroElute® LE RNA Column eliminates most DNA, DNase I digestion is not necessary for most downstream applications. However, certain sensitive RNA applications may require further DNA removal. If an additional DNA removal step is required, please continue to the DNase I Digestion Protocol found on Page 22. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 12.

12. Transfer the MicroElute® LE RNA Column to a new 2 mL Collection Tube.

13. Add 500 µL RWF Wash Buffer.

14. Centrifuge at maximum speed for 30 seconds.

15. Discard the filtrate and reuse the collection tube.

16. Add 500 µL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 8 for instructions.

17. Centrifuge at maximum speed for 30 seconds.

18. Discard the filtrate and reuse the Collection Tube.

19. Repeat Steps 16-18 for a second RNA Wash Buffer II wash step.

20. Centrifuge at maximum speed for 2 minutes to completely dry the MicroElute® LE RNA Column.

    Note: It is important to dry the MicroElute® LE RNA Column matrix before elution. Residual ethanol may interfere with downstream applications.

21. Transfer the MicroElute® LE RNA Column to a clean 1.5 mL microcentrifuge tube.

22. Add 15-20 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the MicroElute® LE RNA Column matrix.

23. Centrifuge at maximum speed for 1 minute.

24. Store eluted RNA at -70°C.

Protocol for Fibrous Tissues

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000*g*
- Vortexer
- Water bath or heat block preset at 55°C
- Nuclease-free pipette tips
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- 100% ethanol
- 3M NaOH
- Sterile deionized water
- Nuclease-free water
- 2-mercaptoethanol (14.3 M)
- Proteinase K (20 mg/mL)
- Optional: RNase-free DNase I Digestion Kit (Cat# E1091)

Before Starting:

- Prepare RNA Wash Buffer II and TRK Lysis Buffer according to the "Preparing Reagents" section on Page 8.
- Optional: Prepare a stock solution of Carrier RNA according to the "Preparing Reagents" section on Page 8.

1. Weigh up to 5 mg tissue and immediately transfer the sample into a nuclease-free 1.5 mL or 2 mL microcentrifuge tube (not provided).

2. Add 150 µL TRK Lysis Buffer.

3. Disrupt the tissue and homogenize lysate using methods described on Page 6-7.

   Note: Incomplete homogenization will cause clogging of the mini column and lead to significantly lower yields. Generally, disruption and homogenization by using a mortar and pestle or needle and syringe can generate lower yield. It is recommended to use a rotor stator homogenizer or bead milling methods for animal tissues.

4. Add 290 µL nuclease-free water and 5 µL Proteinase K (20 mg/mL, not provided). Vortex to mix thoroughly.

5. Incubate at 55°C for 10 minutes.

6. Centrifuge at maximum speed (≥13,000*g*) for 5 minutes at room temperature. A small pellet of tissue debris will form and a thin layer or film can be seen on top of the supernatant.

7. Transfer the supernatant into a new nuclease-free 2 mL microcentrifuge tube.

   Note: Avoid transferring any of the pellet. Hold the pipet tip under the thin layer of film on top of the supernatant, if present. This layer will usually adhere to outside the tip and should not be transferred.

8. Add 0.5 volumes 100% ethanol. Vortex to mix thoroughly.

9. Insert a MicroElute® LE RNA Column into a 2 mL Collection Tube 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.

10. Transfer the entire lysate, including any precipitates that may have formed, from Step 8 to the MicroElute® LE RNA Column.

11. Centrifuge at maximum speed for 15 seconds at room temperature.

12. Discard the filtrate and the collection tube.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the MicroElute® LE RNA Column eliminates most DNA, DNase I digestion is not necessary for most downstream applications. However, certain sensitive RNA applications may require further DNA removal. If an additional DNA removal step is required, please continue to the DNase I Digestion Protocol found on Page 22. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 13.

13. Transfer the MicroElute® LE RNA Column to a new 2 mL Collection Tube.

14. Add 500 µL RWF Wash Buffer.

15. Centrifuge at maximum speed for 30 seconds.

16. Discard the filtrate and reuse the collection tube.

17. Add 500 µL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 8 for instructions.

18. Centrifuge at maximum speed for 30 seconds.

19. Discard the filtrate and reuse the collection tube.

20. Repeat Steps 17-19 for a second RNA Wash Buffer II wash step.

21. Centrifuge at maximum speed for 2 minutes to completely dry the MicroElute® LE RNA Column.

    Note: It is important to dry the MicroElute® LE RNA Column matrix before elution. Residual ethanol may interfere with downstream applications.

22. Transfer the MicroElute® LE RNA Column to a clean 1.5 mL microcentrifuge tube.

23. Add 15-20 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the MicroElute® LE RNA Column matrix.

24. Centrifuge at maximum speed for 1 minute.

25. Store eluted RNA at -70°C.

DNase I Digestion Protocol

Since the HiBind® matrix of the MicroElute® LE RNA Column eliminates most DNA, DNase I digestion is not necessary for most downstream applications. However, certain sensitive RNA applications may require further DNA removal. (See DNase I Digestion Set, Cat# E1091 for further information).

After completing Steps 1-9 of the Laser Dissected Samples Protocol (Pages 9-10), Steps 1-13 of the Microdissected Formalin-fixed Tissues Protocol (Pages 12-14), Steps 1-11 of the Animal Tissue or Cell Cultures Protocol (Pages 16-18), or Steps 1-12 of the Fibrous Tissues Protocol (Pages 20-22), proceed with the following protocol.

User Supplied Material:

- DNase I Digestion Set (E1091)

1. For each MicroElute® LE RNA Column, prepare the DNase I stock solution as follows:

Buffer	Volume per Prep
E.Z.N.A.® DNase I Digestion Buffer	73.5 µL
RNase-free DNase I (20 Kunitz/µL)	1.5 µL
Total Volume	75 µL

Important Notes:

- DNase I is very sensitive and prone to physical denaturing. **Do not vortex the DNase I mixture.** Mix gently by inverting the tube.
- Freshly prepare DNase I stock solution right before RNA isolation.
- Standard DNase buffers are not compatible with on-membrane DNase I digestion. The use of other buffers may affect the binding of RNA to the HiBind® matrix and may reduce RNA yields and purity.
- All steps must be carried out at room temperature. Work quickly, but carefully.

2. Transfer the MicroElute® LE RNA Column to a new 2 mL Collection Tube.

3. Add 250 µL RWF Wash Buffer.

4. Centrifuge at maximum speed for 1 minute.

5. Discard the filtrate and reuse the collection tube.

6. Add 75 µL DNase I digestion mixture directly onto the surface of the membrane of the MicroElute® LE RNA Column.

   Note: Pipet the DNase I directly onto the membrane. DNA digestion will not be complete if some of the mixture is retained on the wall of the MicroElute® LE RNA Column.

7. Let sit at room temperature for 15 minutes.

8. Add 250 µL RWF Wash Buffer to the MicroElute® LE RNA Column.

9. Let sit at room temperature for 2 minutes.

10. Centrifuge at maximum speed for 1 minute.

11. Discard the filtrate and reuse the collection tube.

12. Add 500 µL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 8 for instructions.

13. Centrifuge at maximum speed for 1 minute.

14. Discard the filtrate and reuse the collection tube.

15. Repeat Steps 12-14 for a second RNA Wash Buffer II wash step.

16. Centrifuge at maximum speed for 2 minutes to completely dry the MicroElute® LE RNA Column matrix.

    Note: It is important to dry the MicroElute® LE RNA Column matrix before elution. Residual ethanol may interfere with downstream applications.

17. Transfer the MicroElute® LE RNA Column to a clean 1.5 mL microcentrifuge tube.

18. Add 15-20 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the MicroElute® LE RNA Column matrix.

19. Centrifuge at maximum speed for 1 minute.

20. 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 **1-800-832-8896**.

Problem	Cause	Solution
Little or no RNA eluted	RNA remains on the column	Repeat the elution step.
	Column is overloaded	Reduce the amount of starting material.
	Column Equilibration not performed	Perform the column equilibration protocol as instructed in the manual for consistent results
Problem	Cause	Solution
Clogged column	Incomplete homogenization	Completely homogenize the sample.
		Increase the centrifugation time.
		Reduce the amount of starting material.
Problem	Cause	Solution
Degraded RNA	Starting materials problems	Freeze starting material quickly in liquid nitrogen.
		Do not store tissue culture cells prior to extraction unless they are lysed first.
		Follow protocol closely and work quickly.
	RNase contamination	Ensure not to introduce RNase during the procedure.
		Check buffers for RNase contamination.
Problem	Cause	Solution
Problem in downstream applications	Salt carryover during elution	Ensure RNA Wash Buffer II has been diluted with 100% ethanol as indicated on bottle.
		RNA Wash Buffer II must be stored and used at room temperature.
		Repeat wash steps with RNA Wash Buffer II.
Problem	Cause	Solution
DNA contamination	DNA contamination	Digest with RNase-free DNase and inactivate DNase by incubation at 65°C for 5 minutes in the presence of EDTA.
Problem	Cause	Solution
Low Abs ratios	RNA diluted in acidic buffer or water	Nuclease-free Water is slightly acidic and can lower A_{260}/A_{280} ratios. Use TE buffer to dilute RNA prior to spectrophotometric analysis.

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

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