

# MicroElute® Total RNA Kit

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# Introduction and Overview

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The MicroElute® Total RNA Kit provides a rapid and easy method for the isolation of up to 50 µg of total RNA from small amount of cultured eukaryotic cells, tissues such as laser dissected samples (LDS) or fine needle aspirates (FNA). Normally, up to 5 x 10<sup>5</sup> of eukaryotic cells or 5mg of tissue (amounts depend on the tissue used) 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 method is ready for applications such as RT-PCR\*, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

The MicroElute® Total RNA Kit combines the reversible binding properties of the HiBind® matrix, a new silica-based material with the speed of mini-column spin technology. A specifically formulated high salt buffer system that allows RNA molecules greater than 200 bases to bind to the matrix. Cells or tissues are first lysed under denaturing conditions that practically inactivates RNase. When the ethanol is added, samples are then applied to the HiBind® MicroElute® columns to which total RNA binds, while cellular debris and other contaminants are effectively washed away. High-quality RNA is finally eluted in Nuclease-free Water.

## **New in this Edition:**

- DEPC Water has been replaced with Nuclease-free Water. DEPC Water is no longer provided in this kit.

## Kit Contents

Product	R6831-00	R6831-01	R6831-02
Purifications	5	50	200
MicroElute® RNA Mini 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	1 mL	20 mL	60 mL
Carrier RNA	100 µg	310 µg	1 mg
User Manual	✓	✓	✓

## Storage and Stability

All E.Z.N.A.® Total RNA Kit components are guaranteed for at least 24 months from the date of purchase when stored at room temperature. During shipment, crystals or precipitation may form in the TRK Lysis Buffer. Dissolve by warming buffer to 37°C.

## Important Notes

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### 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 dramatically lower absorbance values. We suggest that you dilute the sample in a buffered solution (TE) for 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

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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 HiBind® RNA Mini 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  $\mu$ L 2-mercaptoethanol per 1 mL TRK Lysis Buffer. This mixture can be stored for 2 weeks at room temperature.

# Homogenization Techniques

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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® RNA Mini 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 and 2 mL Collection Tubes (HCR001, HCR003 and SSI-1370-00)
  - Load the lysate into a homogenizer spin column pre-inserted 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.

# Homogenization Techniques

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## **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  $\mu$ L 2-mercaptoethanol per 1 mL TRK Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.
3. Optimal: Prepare Carrier RNA Stock Solution. Dissolve Carrier RNA in Nuclease-free Water as follows and store in aliquots at  $-70^{\circ}\text{C}$ .

Kit		Starting Material	Add	Concentration
R6831-00	Step 1	Carrier RNA	100 $\mu$ L Nuclease-free Water	1 $\mu\text{g}/\mu\text{L}$
R6831-01			310 $\mu$ L Nuclease-free Water	
R6831-02			1 mL Nuclease-free Water	
	Step 2	5 $\mu$ L Carrier RNA (1 $\mu\text{g}/\mu\text{L}$ )	45 $\mu$ L TRK Lysis Buffer	100 ng/ $\mu$ L
	Step 3	5 $\mu$ L Carrier RNA (1 $\mu\text{g}/\mu\text{L}$ )	95 $\mu$ L TRK Lysis Buffer	5 ng/ $\mu$ L



# Protocol for Laser Dissected Samples

## MicroElute® Total RNA Kit - Protocol for Laser Dissected Samples

### Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000 x g
- Vortexer
- 2-mercaptoethanol (14.3 M)
- 100% ethanol
- 70% ethanol
- Nuclease-free pipette tips
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- 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
- 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.

# Protocol for Laser Dissected Samples

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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® RNA Mini Column into 2 mL Collection Tube (provided).
7. Transfer the lysate from Step 5, including any precipitates that may have formed, to the MicroElute® RNA Mini Column.
8. Centrifuge at maximum speed ( $\geq 13,000 \times g$ ) 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® RNA Mini 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 RNA 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® RNA Mini Column to a new 2 mL Collection Tube.
11. Add 500  $\mu$ L RWF Wash Buffer.
12. Centrifuge at maximum speed for 30 seconds.
13. Discard the filtrate and reuse the Collection Tube.

# Protocol for Laser Dissected Samples

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14. Add 500  $\mu$ L RNA Wash Buffer II.

**Note:** RNA Wash Buffer II must be diluted with 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<sup>®</sup> RNA Mini Column.

**Note:** It is important to dry the MicroElute<sup>®</sup> RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

19. Transfer the MicroElute<sup>®</sup> RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

20. Add 15-20  $\mu$ L Nuclease-free Water.

**Note:** Make sure to add water directly onto the MicroElute<sup>®</sup> RNA Mini Column matrix.

21. Centrifuge at maximum speed for 1 minute.

22. Store eluted RNA at -70°C.

# Protocol for Microdissected Formalin-Fixed Tissues

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## MicroElute® Total RNA Kit - 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 300nt. Since the Total RNA isolation protocol will remove most fragments less than 200nt. 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 x *g*
- Vortexer
- Water bath or heat block capable of 55°C
- 100% ethanol
- 2-mercaptoethanol (14.3 M)
- Proteinase K (20 mg/mL)
- Nuclease-free pipette tips
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- 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 4µL (20ng) 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.

# Protocol for Microdissected Formalin-Fixed Tissues

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3. Adjust the volume to 150  $\mu\text{L}$  with TRK Lysis Buffer.
4. Add 295  $\mu\text{L}$  Nuclease-free Water.
5. Add 5  $\mu\text{L}$  Proteinase K (20 mg/mL).
6. Incubate at 55°C for 10 minutes.
7. Centrifuge at maximum speed ( $\geq 13,000 \times g$ ) 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 ( $\sim 450 \mu\text{L}$ ) to a new nuclease-free 1.5 mL or 2 mL microcentrifuge tube (not supplied).  
  
**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 to the cleared lysate.
10. Insert a MicroElute® RNA Mini Column into 2 mL Collection Tube.
11. Transfer the entire lysate, including any precipitates that may have formed, from Step 9 to the MicroElute® RNA Mini Column.
12. Centrifuge at maximum speed for 15 seconds at room temperature.
13. Discard the filtrate and reuse the Collection Tube.

# Protocol for Microdissected Formalin-Fixed Tissues

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**Optional:** This is the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the MicroElute® RNA Mini 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 RNA 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® RNA Mini Column to a new 2 mL Collection Tube (provided).

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 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® RNA Mini Column.

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

# Protocol for Microdissected Formalin-Fixed Tissues

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23. Transfer the MicroElute® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

24. Add 15-20  $\mu$ L Nuclease-free Water.

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

25. Centrifuge at maximum speed for 1 minute.

26. Store eluted RNA at -70°C.

# Protocol for Animal Tissue or Cell Cultures

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## MicroElute® Total RNA Kit - 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 x *g*
- Vortexer
- 100% ethanol
- 70% ethanol
- 2-mercaptoethanol (14.3 M)
- Nuclease-free pipette tips
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- 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 sample. Do not use more than 5mg tissue or  $5 \times 10^5$  of cells.

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

**Optional:** If using  $<5,000$  cells, add 4 $\mu$ L (20ng) Carrier RNA Stock Solution before homogenization.

2. Add 350 $\mu$ L of TRK Lysis Buffer to a 1.5mL or 2mL microcentrifuge tube(not provided).

**Note:** Add 20  $\mu$ L 2-mercaptoethanol per 1 mL TRK Lysis Buffer before use. Please see Page 8 for instructions.



# Protocol for Animal Tissue or Cell Cultures

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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 ( $\geq 13,000 \times g$ ) for 2 minutes.
6. Transfer the cleared supernatant to a new microcentrifuge tube.
7. Add an 1 volume of 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® RNA Mini Column into 2 mL Collection Tube.
9. Transfer the entire lysate, including any precipitates that may have formed, from Step 7 to the MicroElute® RNA Mini Column.
10. Centrifuge at maximum speed for 15 seconds at room temperature.
11. Discard the filtrate and reuse the collection tube.

**Optional:** This is the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the MicroElute® RNA Mini 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 RNA 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® RNA Mini Column to a new 2 mL Collection Tube.

# Protocol for Animal Tissue or Cell Cultures

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13. Add 500  $\mu$ L RWF Wash Buffer.
14. Centrifuge at maximum speed for 30 seconds.
15. Discard the filtrate and reuse the collection tube.
16. Add 500  $\mu$ L RNA Wash Buffer II.  
**Note:** RNA Wash Buffer II must be diluted with 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<sup>®</sup> RNA Mini Column.  
**Note:** It is important to dry the MicroElute<sup>®</sup> RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.
21. Transfer the MicroElute<sup>®</sup> RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).
22. Add 15-20  $\mu$ L Nuclease-free Water.  
**Note:** Make sure to add water directly onto the MicroElute<sup>®</sup> RNA Mini Column matrix.
23. Centrifuge at maximum speed for 1 minute.
24. Store eluted RNA at  $-70^{\circ}\text{C}$ .

# Protocol for Fibrous Tissues

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## MicroElute® Total RNA Kit - Protocol for Fibrous Tissues

### Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000 x *g*
- Vortexer
- Water bath or heat block preset at 55°C
- 100% ethanol
- Nuclease-free water
- 2-mercaptoethanol (14.3 M)
- Proteinase K (20 mg/mL)
- Nuclease-free pipette tips
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- 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 5mg tissue and immediately transfer the sample into a nuclease-free 1.5mL or 2mL microcentrifuge tube.
2. Add 150µl 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.

# Protocol for Fibrous Tissues

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4. Add 290 $\mu$ L nuclease-free water and 5 $\mu$ L Proteinase K (mg/mL, not provided).
5. Incubate at 55°C for 10 minutes.
6. Centrifuge at maximum speed ( $\geq 13,000 \times g$ ) 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 volume 100% ethanol. Vortex to mix thoroughly.
9. Insert a MicroElute<sup>®</sup> RNA Mini Column into 2 mL Collection Tube.
10. Transfer the entire lysate, including any precipitates that may have formed, from Step 8 to the MicroElute<sup>®</sup> RNA Mini Column.
11. Centrifuge at maximum speed for 15 seconds at room temperature.
12. Discard the filtrate and reuse the collection tube.

**Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol.** Since the HiBind<sup>®</sup> matrix of the MicroElute<sup>®</sup> RNA Mini 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 RNA 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.

# Protocol for Fibrous Tissues

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13. Transfer the MicroElute® RNA Mini 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 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® RNA Mini Column.

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

22. Transfer the MicroElute® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

23. Add 15-20 µL Nuclease-free Water.

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

24. Centrifuge at maximum speed for 1 minute.

25. Store eluted RNA at -70°C.

# DNase I Digestion Protocol

## E.Z.N.A.® Total RNA Kit I - DNase I Digestion Protocol

Since the HiBind® matrix of the RNA Mini 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 Tissue Protocol (Pages 12-13), Steps 1-11 of the Animal Tissue or Cell Cultures Protocol (Pages 16-17), or Steps 1-12 of the Fibrous Tissues Protocol (Pages 19-20), proceed with the following protocol.

### User Supplied Material:

- DNase I Digestion Set (E1091)
1. For each MicroElute® RNA Mini 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® RNA Mini Column to a 2 mL Collection Tube.

# DNase I Digestion Protocol

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3. Add 250  $\mu$ L RWF Wash Buffer.
4. Centrifuge at maximum speed for 1 minute.
5. Discard the filtrate and reuse the collection tube.
6. Add 75  $\mu$ L DNase I digestion mixture directly onto the surface of the membrane of the MicroElute<sup>®</sup> RNA Mini 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<sup>®</sup> RNA Mini Column.
7. Let sit at room temperature for 15 minutes.
8. Add 250  $\mu$ L RWF Wash Buffer to the MicroElute<sup>®</sup> RNA Mini 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  $\mu$ L RNA Wash Buffer II.  
  
**Note:** RNA Wash Buffer II must be diluted with 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.

# DNase I Digestion Protocol

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

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

17. Transfer the MicroElute® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

18. Add 15-20  $\mu$ L Nuclease-free Water.

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

19. Centrifuge at maximum speed for 1 minute.

20. Store eluted RNA at  $-70^{\circ}\text{C}$ .



## 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.
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 culture 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 acidic and can dramatically lower Abs <sub>260</sub> values. Use TE Buffer to dilute RNA prior to spectrophotometric analysis.

## Ordering Information

The following components are available for purchase separately.  
(Call Toll Free at 1-800-832-8896)

Product	Part Number
TRK Lysis Buffer (100 mL)	PR021
RNA Wash Buffer II (25 mL)	PR031
Nuclease-free Water (100 mL)	PR032
RNase-free DNase Set (50 preps)	E1091-01
RNase-free DNase Set (200 preps)	E1091-02
Omega Homogenizer Columns (200 preps)	HCR003
Proteinase K (>600 mAU/mL, Solution; 2 mL)	AC115
Proteinase K (>600 mAU/mL, Solution; 10 mL)	AC116
RNA-Lock Stabilizer Reagent (50 mL)	R0424-01
RNA-Lock Stabilizer Reagent (250 mL)	R0424-02

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