E.Z.N.A.® MicroElute® Total RNA Kit

Revision No: 3.0

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

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

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 according to the instructions on the bottle.
- Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer.
- Optional: Prepare a stock solution of Carrier RNA according to the table below.

Optional: Prepare Carrier RNA Stock Solution as follows and store in aliquots at -70°C.

Kit		Starting Material	Add	Concentration
R6831-00 R6831-02	Step 1	Carrier RNA (vial)	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

RNA Purification from Laser Dissected Samples

LYSE

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

   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.
3. Adjust the volume to 350 µL with TRK Lysis Buffer. Vortex for 30 seconds to mix thoroughly.
4. 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.

BIND

5. 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.

BIND

6. Transfer the entire lysate from Step 4, including any precipitates that may have formed, to the MicroElute® LE RNA Column.
7. Centrifuge at maximum speed (≥13,000*g*) for 15 seconds at room temperature. Discard the filtrate and 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® 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 of the product manual. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 8.

WASH

8. Transfer the MicroElute® LE RNA Column to a new 2 mL Collection Tube.
9. Add 500 µL RWF Wash Buffer. Centrifuge at maximum speed for 30 seconds. Discard the filtrate and reuse the collection tube.
10. Add 500 µL RNA Wash Buffer II diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 30 seconds. Discard the filtrate and reuse the collection tube.
11. Repeat Step 10 for a second RNA Wash Buffer II wash step.
12. 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.

ELUTE

13. Transfer the MicroElute® LE RNA Column to a clean 1.5 mL microcentrifuge tube.
14. Add 15-20 µL Nuclease-free Water directly onto the MicroElute® LE RNA Column matrix. Centrifuge at maximum speed for 1 minute.
15. Store eluted RNA at -70°C.

RNA Purification from Animal Tissue or Cell Cultures

Optional: Prepare Carrier RNA Stock Solution according to the table on the reverse page and store in aliquots at -70°C.

LYSE

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. 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.
3. Transfer homogenized sample to the microcentrifuge tube containing TRK Lysis Buffer. Vortex for 30 seconds to mix thoroughly.
4. Centrifuge at maximum speed (≥13,000*g*) for 2 minutes.

BIND

5. Transfer the cleared supernatant to a new microcentrifuge tube.
6. Add 1 volume 70% ethanol. Vortex to mix thoroughly. Do not centrifuge. A precipitate may form at this point. This will not interfere with the RNA purification.
7. Proceed to Step 5 of the RNA PURIFICATION FROM LASER DISSECTED SAMPLES protocol on the reverse page.