E.Z.N.A.® TOTAL RNA KIT I

Revision No: 3.1

Quick Guide

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

Product	R6834-00	R6834-01	R6834-02	R6834-03
Purifications	5	50	200	600
HiBind® RNA Mini Columns	5	50	200	600
2 mL Collection Tubes	10	100	400	1200
TRK Lysis Buffer	5 mL	40 mL	150 mL	500 mL
RNA Wash Buffer I	5 mL	50 mL	200 mL	200 mL
RNA Wash Buffer II	5 mL	12 mL	50 mL	200 mL
Nuclease-free Water	2 mL	30 mL	60 mL	125 mL

Supplied by user:

- Microcentrifuge capable of at least 14,000*g*
- RNase-free pipette tips and 1.5 mL microcentrifuge tubes
- 100% ethanol
- 70% ethanol
- Homogenization equipment
  - Omega Homogenizer Columns (HCR003)
  - Needle and Syringe
  - Mortar and pestle
  - Glass Beads
  - Rotor-stator Homogenizer
- Optional: 14.3M 2-mercaptoethanol (β-ME)

Before starting:

- Optional: Add 20 µL β-ME per 1 mL TRK Lysis Buffer.
- Prepare RNA Wash Buffer II according to the directions on the bottle

RNA Extraction and Purification from Tissue

LYSE

1. Determine the proper amount of starting material. Homogenize and disrupt the tissue according to the table below. For homogenization techniques, please refer to the downloadable product manual from www.omegabiotek.com.

Amount of Tissue	Amount of TRK Lysis Buffer
≤ 15 mg	350 µL
20-30 mg	700 µL

Note: For samples stored in RNALater® use 700 µL TRK Lysis Buffer.
Optional: Add 20 µL β-ME per 1 mL TRK Lysis Buffer. Store for up to 4 weeks at room temperature.

2. Centrifuge at maximum speed (≥12,000*g*) for 5 minutes.
3. Transfer the cleared supernatant to a clean 1.5 mL microcentrifuge tube (not supplied). Do not transfer any fatty upper layer that may have formed as it may reduce RNA yield or clog the column.

BIND

4. Add 1 volume 70% ethanol. Vortex to mix thoroughly. Do not centrifuge. If any sample has lost its volume during homogenization, adjust the volume of ethanol accordingly.
5. Insert a HiBind® RNA Mini Column into a 2 mL Collection Tube.
6. Transfer 700 µL sample (including any precipitate that may have formed) to the HiBind® RNA Mini Column.
7. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and reuse the Collection Tube.
8. Repeat Steps 6-7 until all of the sample has been transferred to the column.

OPTIONAL: This the starting point of an optional on-membrane DNase I Digestion protocol. If an RNA removal step is required, please continue to the DNase I Digestion Protocol on the reverse page. (See DNase I Digestion Set, Cat# E1091 for more information). If DNase I digestion is not required, proceed to Step 9.

WASH

9. Add 500 µL RNA Wash Buffer I. Centrifuge at 10,000*g* for 30 seconds. Discard the filtrate and reuse the collection tube.
10. Add 500 µL RNA Wash Buffer II diluted with 100% ethanol (see bottle for instructions). Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and reuse the collection tube.
11. Repeat Step 10 for a second RNA Wash Buffer II wash step.
12. Centrifuge the empty HiBind® RNA Mini Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

ELUTE

13. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).
14. Add 40-70 µL Nuclease-free Water. Centrifuge at maximum speed for 2 minutes. Store eluted RNA at -70°C.

RNA Extraction and Purification from Cultured Cells

LYSE

1. Determine the proper amount of starting material. Harvest and disrupt cells (do not use more than 1 x 10^7 cells) with TRK Lysis Buffer according to the table below. Vortex or pipet up and down to mix thoroughly. For cell harvesting techniques, please refer to the downloadable product manual from www.omegabiotek.com.

Number of Cells	Amount of TRK Lysis Buffer
< 5 x 10^6	350 µL
5 x 10^6 - 1 x 10^7	700 µL

Optional: Add 20 µL β-ME per 1 mL TRK Lysis Buffer. Store for up to 4 weeks at room temperature.

2. Homogenize the cells accordingly to one of the following methods:

- Syringe and Needle: Shear high MW DNA by passing the lysate through a narrow needle (19-21 gauge) 5-10 times.
- Homogenizer Mini Column (HCR003): Load the lysate into a Homogenizer Mini Column inserted into a 2 mL Collection Tube. Centrifuge at maximum speed (≥12,000*g*) for two minutes to collect the homogenized lysate.

Note: Incomplete homogenization of the sample may cause the column to clog resulting in decreased yields.

3. Proceed to Step 4 of the RNA EXTRACTION AND PURIFICATION FROM TISSUE protocol on the reverse page.

DNase I Digestion Protocol

1. For each HiBind® RNA Mini Column, prepare 75 µL DNase I stock solution (73.5 µL DNase I Digestion Buffer + 1.5 µL DNase I (20 Kunitz/µL)). See DNase I Digestion Set, Cat# E1091 for more information.
2. Add 250 µL RNA Wash Buffer I. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and reuse the Collection Tube.
3. Add 75 µL DNase I digestion mixture directly onto the surface of the membrane of the HiBind® RNA Mini Column. DNA digestion will not be complete if some of the mixture is retained on the wall of the HiBind® RNA Mini Column.
4. Let sit at room temperature for 15 minutes.
5. Add 250 µL RNA Wash Buffer I. Let sit at room temperature for 2 minutes.
6. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and reuse the Collection Tube.
7. Proceed to Step 10 of the RNA EXTRACTION AND PURIFICATION FROM TISSUE protocol above.