E.Z.N.A.® HP Total RNA Kit

Revision No: 1.1

Quick Guide

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

Product	R6812-00	R6812-01	R6812-02
Purifications	5	50	200
HiBind® RNA Mini Columns	5	50	200
RNA Homogenizer Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
GTC Lysis Buffer	5 mL	40 mL	150 mL
RNA Wash Buffer I	5 mL	50 mL	200 mL
RNA Wash Buffer II	5 mL	12 mL	50 mL
Nuclease-Free Water	2 mL	15 mL	60 mL

Supplied by user:

- Microcentrifuge capable of at least 14,000 x *g*
- RNase-free pipette tips and 1.5 mL microcentrifuge tubes
- 100% ethanol
- 70% ethanol in sterile DEPC-treated water
- Vortexer
- Disruption equipment such as glass beads or rotor-stator homogenizer
- 14.3M 2-mercaptoethanol (β-ME)
- Optional: DNase I (Cat#E1091) for DNase I Digestion Protocol

Before starting:

- Add 20 µL β-ME per 1 mL GTC Lysis Buffer. Store at RT for 4 weeks.
- Prepare RNA Wash Buffer II according to the directions on the bottle.

RNA Extraction and Purification from Animal Cells

LYSE

1. Determine the proper amount of starting material. Harvest and disrupt the cells in GTC Lysis Buffer mixed with β-ME according to the table below. Vortex to mix thoroughly. For harvesting techniques, please refer to the downloadable product manual from www.omegabiotek.com.

Number of Cells	Amount of GTC Lysis Buffer
< 5 x 10^6	350 µL
5 x 10^6 - 1 x 10^7	700 µL
Dish Diameter (cm)	**Amount of GTC Lysis Buffer (µL)**
< 6	350 µL
6-10	700 µL

BIND

2. Insert a RNA Homogenizer Mini Column into a 2 mL Collection Tube.
3. Transfer the lysate to the RNA Homogenizer Mini Column. Centrifuge at 13,000 x *g* for 1 minute. Save the filtrate and discard the RNA Homogenizer Mini Column.
4. Add 1 volume 70% ethanol. Vortex to mix thoroughly. Do not centrifuge. If any sample has lost 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. Centrifuge at 10,000 x *g* for 1 minute. Discard the filtrate and reuse the Collection Tube.
7. Repeat Step 6 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 a DNA removal is required, please continue to the DNase I Digestion Protocol at the bottom of this page. (See DNase I Digestion Set, Cat# E1091 for more information). If DNase I digestion is not required, proceed to Step 8.

WASH

8. Add 500 µL RNA Wash Buffer I. Centrifuge at 10,000 x *g* for 30 seconds. Discard the filtrate and reuse the collection tube.
9. Add 500 µL RNA Wash Buffer II diluted with 100% ethanol (see bottle for instructions). Centrifuge at 10,000 x *g* for 1 minute. Discard the filtrate and reuse the collection tube.
10. Repeat Step 9 for a second RNA Wash Buffer II wash step.
11. 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

12. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube.
13. 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 Animal Tissue

LYSE

1. Determine the proper amount of starting material. Disrupt the tissue in GTC Lysis Buffer mixed with β-ME according to the table below using either a rotor-stator homogenizer or bead milling. For homogenization techniques, please refer to the downloadable product manual from www.omegabiotek.com.

Amount of Tissue	Amount of GTC Lysis Buffer (µL)
≤ 15 mg	350 µL
20-30 mg	700 µL

Note: For samples stored in RNALater® use 700 µL GTC Lysis Buffer.

2. Centrifuge at maximum speed for 5 minutes.

   Note: In some preparations, a fatty upper layer will form after centrifugation. Transfer of any of the fatty upper layer may reduce RNA yield or clog the column.

3. Proceed to Step 2 of the RNA EXTRACTION AND PURIFICATION FROM ANIMAL CELLS protocol on the reverse page.

DNase I Digestion Protocol

DNase I Preparation (See DNase I Digestion Set, Cat# E1091 for more information):

Buffer	Volume per Sample
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

1. Complete Steps 1-7 of the RNA EXTRACTION AND PURIFICATION FROM ANIMAL CELLS or Steps 1-3 of the ANIMAL TISSUE protocol before beginning.
2. Add 250 µL RNA Wash Buffer I. Centrifuge at 10,000 x *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. Centrifuge at 10,000 x *g* for 1 minute. Discard the filtrate and reuse the collection tube.
6. Proceed to Step 9 of the RNA EXTRACTION AND PURIFICATION FROM CULTURED ANIMAL CELLS protocol above.