

## Contents

Introduction.....	2
Principle.....	2
Storage.....	2
Kit Contents.....	3
Important Notes.....	3
Before Starting.....	4
E-Z 96® Total RNA Spin Protocol.....	4
E-Z 96® Total RNA Vacuum Protocol.....	7
Quantitation and Storage of RNA.....	10
Clean the 2 ml Square Plate.....	10
Troubleshooting Tips.....	12

**Revised September 2007**

## Introduction

E-Z 96® Total RNA Isolation Kits are designed for isolation of Total RNA from culture cells. RNA purified using the E-Z 96® Total RNA method is ready for applications such as RT-PCR\*, qPCR\*, differential display, microarrays, etc. DNA purified from this kit is suitable for PCR, Souther blot, genotyping and ANP analysis.

## Principle

The E-Z 96® Total RNA isolate kits combines reversible binding properties of the HiBind® RNA technology with a specially designed buffer system which selective binds RNA. Samples are first lysed and homogenized in a specially designed denature buffer (GTC), which immediately inhibits the activity of RNase. The Lysate is then added ethanol to create proper RNA binding condition, the sample is then loaded into the E-Z 96® RNA Plate to bind RNA. With a brief centrifugation or vacuum, the samples pass through the plate and the RNA binds to the Hibind™ matrix. After two wash steps, purified RNA is eluted with RNase-free water.

## Storage and Stability

All components in the E-Z 96® Total RNA should be stored at room temperature. During shipping and storage in cool ambient conditions, crystals may form in the TRK Lysis Buffer. Simply warm the buffer to 37°C and gently shake its container to dissolve. All kit components are guaranteed for at least 12 months from date of purchase.

\*The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 (and international equivalents) owned by Hoffmann-LaRoche, Inc.

## Kit Contents

E-Z 96® Total RNA Kits	R1034-00	R1034-01	R1034-02
E-Z 96® MicroElute RNA Plates	1	4	20
Square-Well Collection Plate*	1*	4*	20*
Racked Microtubes (1.2ml)	1 x 96	4 x 96	20x 96
8-Strip Microtube Caps	12 x 8	48 x 8	240 x 8
Aera Sealing Film	6	24	120
TRK Lysis Buffer	30 ml	120 ml	600 ml
RWC Wash Buffer	90 ml	2 x 180 ml	2 x 700 ml
RNA Wash Buffer II	40 ml	3 x 50 ml	3 x 200 ml
DEPC-ddH <sub>2</sub> O	20 ml	60 ml	250 ml
Instruction Manual	1	1	1

\* 2 ml Square-well plates are reusable. See Page 11 for cleaning instructions.

## Important Notes

Please take a few minutes to read this booklet thoroughly to become familiar with the protocol. Prepare all materials required before starting the procedure to minimize RNA degradation.

- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.
- Carefully apply the sample or solution to the HiBind® RNA membrane. Avoid touching the membrane with pipet tips.
- Dilute **RNA Wash Buffer II** with **absolute ethanol** before use, and store at room temperature.

R1034-00	Add 160 ml 96%-100% ethanol.
R1034-01	Add 200 ml 96%-100% ethanol per bottle.
R1034-02	Add 800 ml 96%-100% ethanol per bottle.

## E-Z 96<sup>®</sup> Total RNA Protocol with Centrifugation

### Materials supplied by user

- 96%-100% ethanol
- 70% ethanol
- Multichannel pipet
- RNase-free filter pipette tips
- Reagent reservoirs for multichannel pipets
- Centrifuge with rotor for 96-well plates
- Disposable latex gloves

**Note:** All steps must be carried out at room temperature. Work carefully, but quickly.

### Procedure:

1. **A. LYSIS OF MONOLAYER CULTURED CELLS GROWN IN A MULTI-WELL TISSUE CULTURE PLATE:** Remove the medium completely by pipetting. Add 150  $\mu$ l of TRK Lysis Buffer directly to each well.  
  
**B. LYSIS OF SUSPENSION CULTURED CELLS:** Transfer aliquots of up to  $5 \times 10^5$  cells into the wells of a 96-well microplate. Spin the plate at  $300 \times g$  for 5 minutes. Remove the medium completely by pipetting. Add 150 $\mu$ l of TRK Lysis Buffer directly to each well.
2. Keep the microplate flat on the bench. Shake vigorously, end to end and side to side, for a total of 30 seconds.
3. Incubate at room temperature for 10 minutes.
4. Add one volume (150  $\mu$ l) of 70% ethanol to the lysate; mix thoroughly by pipetting up and down 5 to 10 times. Do no centrifuge.
5. Place the E-Z 96<sup>®</sup> RNA plate atop the 2 ml deep-well plate and carefully transfer entire sample from Step 4 (including any precipitate) to each well of the E-Z 96<sup>®</sup> RNA plate.
6. Seal the E-Z 96<sup>®</sup> RNA plate with Aera Sealing Film. Load the E-Z 96<sup>®</sup> RNA /2

ml square-well plate into a microplate holder, and place the whole assembly into the rotor bucket of the centrifuge. Spin at  $5,000 \times g$  for 5 minutes at room temperature. Remove the Aera Sealing Film and discard the flow-through.

7. (Optional) Add 50ul DNase Digestion Mix (48.5ul DNase Buffer and 1.5ul DNase) to the well of E-Z 96 RNA Plate. Incubate at room temperature for 15 minutes to digest DNA.
8. Add 700 $\mu$ l of RWC Wash Buffer directly into the each well of the E-Z 96<sup>®</sup> RNA plate, Seal the plate with new Aera Sealing Film. (if DNase add, incubate at room temperature for 5 minutes.) Centrifuge at  $5,000 \times g$  for 5 minutes at room temperature.
9. Remove the Aera Sealing Film and add 700  $\mu$ l Wash Buffer II diluted with ethanol to each well of the E-Z 96<sup>®</sup> RNA plate. Seal the plate with new Aera Sealing Film. Centrifuge at  $5,000 \times g$  for 5 minutes at room temperature. Discard the flow-through and re-use the Square-Well Collection Plate.

**Note: Wash Buffer II Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.**

10. Remove the Aera Sealing Film. Add another 700  $\mu$ l of RNA Wash Buffer II to each well of E-Z 96<sup>®</sup> RNA plate. Seal the plate with new Aera Sealing Film. Centrifuge at  $5,000 \times g$  for 10 minutes at room temperature. The prolonged centrifugation is necessary to dry the E-Z 96<sup>®</sup> RNA plate.

**Note: It is very important to dry the E-Z 96<sup>®</sup> RNA plate completely before the elution step to remove residual ethanol that might otherwise interfere with downstream applications.**

11. Elution of RNA: Remove the sealing film and place the E-Z 96<sup>®</sup> RNA plate onto the microtube rack containing 1.2 ml microtubes (supplied with kit).
12. Add 40-70 $\mu$ l of DEPC-treated water to each well, and seal the E-Z 96<sup>®</sup> RNA plate with new Aera Sealing Film(supplied with kit). Make sure to add water directly onto the RNA matrix. Incubate for 3 minutes at room temperature. Centrifuge at  $5,000 \times g$  for 5 minutes at room temperature to elute RNA.
13. Remove the Aera Sealing Film. Repeat Steps 12 and 13 for second elution.

**Note:** Elution volume and numbers can vary according to user preference. To maintain a higher RNA concentration, first eluate can be used for second elution.

## E-Z 96<sup>®</sup> Total RNA Vacuum Protocol

### Materials supplied by user

- 96-100% ethanol
- 70% ethanol
- Multichannel pipets
- RNase-free filter pipette tips
- Reagent reservoirs for multichannel pipets
- Vacuum manifold (Product# Vac-03)
- Vacuum source capable of generating a vacuum pressure of -900 mbar
- Disposable latex gloves
- 2ml 96-well deep-well plate
- 800 µl microplate

**Note:** All steps must be carried out at room temperature. Work quickly, but carefully. Become familiar with the manifold by reading the manufacturer's instructions before starting this vacuum protocol.

### Procedure:

1. A. LYSIS OF MONOLAYER CULTURED CELLS GROWN IN A MULTI-WELL TISSUE CULTURE PLATE: Remove the medium completely by pipetting. Add 150ul of TRK Lysis Buffer directly to each well.  
  
B. LYSIS OF SUSPENSION CULTURED CELLS: Transfer aliquots of up to  $5 \times 10^5$  cells into the wells of a 96-well microplate. Spin the plate at  $300 \times g$  for 5 minutes. Remove the medium completely by pipetting. Add 150 ul of TRK Lysis Buffer directly to each well.
2. Keep the microplate flat on the bench. Shake vigorously, end to end and side to side, for a total of one minute.
3. Incubate at room temperature for 10 minutes.
4. Add one volume (150µl) of 70% ethanol to the sample; mix thoroughly by

pipetting up and down 3 to 4 times.

5. **PREPARE THE VACUUM MANIFOLD:** Place the 2ml Square-Well Collection Plate inside the vacuum manifold base. Place the manifold's top section squarely over its base. Place the E-Z 96<sup>®</sup> RNA Plate on the manifold's top section, making sure the E-Z 96<sup>®</sup> RNA Clearance Plate is seated tightly on the rubber ring. Connect the vacuum manifold to the vacuum source. Keep the vacuum switch off.
6. **Carefully transfer the entire sample from Step 4 to each well of the E-Z 96<sup>®</sup> RNA plate.** Switch on the vacuum source. Apply vacuum until all of the sample contents passes through the well membranes.
7. (Optional) Add 50ul DNase Digestion Mix (48.5ul DNase Buffer and 1.5ul DNase) to the well of E-Z 96 RNA Plate. Incubate at room temperature for 15 minutes to digest DNA.
8. **Add 700µl of RWC Wash Buffer directly into each well of the E-Z 96<sup>®</sup> RNA plate.** (if DNase add, Incubate at room temperature for 5 minutes.) Apply the vacuum until the solution is completely passed through. Switch off the vacuum, and ventilate the manifold.
9. **Add 700µl of RNA Wash Buffer II to each well of the of E-Z 96<sup>®</sup> RNA plate and apply the vacuum until transfer is complete.** Switch off the vacuum.  
  
*Note: Wash Buffer II Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.*
10. **Add another 700µl of RNA Wash Buffer II to each well of the of E-Z 96<sup>®</sup> RNA plate and apply the vacuum until transfer is complete.** Switch off the vacuum *and* ventilate the manifold.
11. Remove the E-Z 96<sup>®</sup> RNA plate from the top plate of the vacuum manifold, and strike the bottom of the E-Z 96<sup>®</sup> RNA plate on a stack of paper towels. Repeat a few times until there is no liquid released onto the paper towels.
12. **Place the E-Z 96<sup>®</sup> RNA plate back to the top plate of the manifold. Apply vacuum for 15 minutes.** Turn off the vacuum and ventilate the manifold.
13. Replace the Square-Well Collection Plate or waste collection tray with a

microtube rack containing the 1.2ml microtubes. Reassemble the manifold. Place the E-Z 96<sup>®</sup> RNA plate on the top plate of the manifold.

14. **Elution RNA: Add 40-70 µl of DEPC-treated water to each well, and seal E-Z 96<sup>®</sup> RNA plate with new sealing film (supplied with kit).** Make sure to add water directly onto RNA matrix. Incubate for 1 minute at room temperature. Switch on the vacuum source for 5 minutes. Switch off the vacuum and ventilate the manifold.
15. Repeat the elution with a second volume of 40-70 µl DEPC-treated water.  
  
*Note: Elution volume and number can vary according to user preference. To maintain a higher RNA concentration, first eluate can be used for second elution.*
16. Remove the eluted RNA from vacuum manifold and store at -80°C.

## Quantitation and Storage of RNA

To determine the concentration and purity of RNA, measure absorbency at 260 nm and 280 nm in a spectrophotometer. 1 O.D. unit measured at 260 nm corresponds to 40 µg of RNA per ml. The ratio of  $A_{260}/A_{280}$  of pure nucleic acids is 2.0, while for pure protein it is approximately 0.6. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. (Phenol has an absorbency maximum at 275 nm and can interfere with spectrophotometric analysis of DNA or RNA. However, the HiBind® RNA technology eliminates the use of phenol and avoids this problem.) Store RNA samples at -80°C in water. Under such conditions RNA is stable for more than a year.

### Clean the 2ml deep well plates:

2ml deep well plates are supplied with each kit. If extra plates are needed, please call our customer service department for ordering information. To reuse the deep well plates, rinse them thoroughly with tap water, incubate overnight in 0.2M NaOH/1mM EDTA, rinse with distilled water and dry by air.

## Troubleshooting Tips

Problem	Cause	Suggestion
Little or no RNA eluted	Add Carrier RNA to TRK Lysis Buffer	<ul style="list-style-type: none"> <li>Dissolve the carrier RNA with TRK Lysis Buffer and repeat the purification with new sample.</li> <li>Repeat elution.</li> <li>Pre-heat DEPC-water to 60° C prior to elution.</li> <li>Incubate for 5 min with water prior to elution</li> </ul>
	RNA remains on the plate	<ul style="list-style-type: none"> <li>Incubate for 5 min with water prior to elution</li> </ul>
Problem in downstream applications	Plate is overloaded	<ul style="list-style-type: none"> <li>Reduce quantity of starting material.</li> </ul>
	Salt carry-over during elution	<ul style="list-style-type: none"> <li>Ensure RNA Wash Buffer II has been diluted with 4 volumes of 100% ethanol as indicated on bottle.</li> <li>1 X Wash Buffer II must be stored at room temperature.</li> <li>Repeat wash with Wash Buffer II.</li> </ul>
DNA contamination	Inhibitors of PCR	<ul style="list-style-type: none"> <li>Use less starting material</li> <li>Prolong incubation with Buffer GTC to completely lyse cells</li> </ul>
	Too much starting material	<ul style="list-style-type: none"> <li>Reduce the cell number and repeat the extraction.</li> <li>Perform DNase digestion</li> </ul>
Little or no RNA eluted	Lost RNA during process	<ul style="list-style-type: none"> <li>Make sure RNA wash Buffer is diluted with absolute ethanol with correct amount of ethanol</li> </ul>