Contents

Revised September 2007

Introduction

E-Z 96® DNA/RNA Isolation Kits are designed for isolation of cellular RNA and genomic DNA simultaneously from single biological sample. Lysates are first passed through a E-Z 96® DNA binding plate to selectively isolate DNA and then through a E-Z 96® RNA plate to selectively isolate RNA. Pure DNA and RNA are isolated from entire sample. Unlike some of other procedures that either the sample or purified nucleic acid is divided sample into two parts before being processed separately.

RNA purified using the E-Z 96® DNA/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 analysises.

Principle

The E-Z 96® DNA/RNA isolate kits combines reversible binding properties of HiBind® RNA technology with a a specially designed buffer system which selective bind DNA to a DNA plate before RNA isolation. Samples are first lysed and homogenized in a specially designed denature buffer (GTC), which immediately inhibit the activity of RNase and DNase. The lyste is then passed through a E-Z 96® DNA Plate which will selectively bind genomic DNA. After two quick wash steps, the purified DNA is eluted from E-Z 96® DNA Plate. The flow-through lysate from E-Z 96® DNA Plate 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

All components in the E-Z 96® DNA/RNA Kit should be stored at room temperature. During shipping and storage in cool ambient conditions, crystals may form in the GTC 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® DNA/RNA Kits	R6732-00	R6732-01	R6732-02
E-Z 96® DNA Plates	1	4	12
E-Z 96® RNA Plates	1	4	12
Square-Well Collection Plate*	1*	2*	4*
Racked Microtubes (1.2ml)	2 x 96	8 x 96	24x 96
8-Strip Microtube Caps	24 x 8	96 x 8	288 x 8
Aera Sealing Film	8	32	96
GTC Lysis Buffer	30 ml	125 ml	400 ml
RNA Wash Buffer I	60 ml	250 ml	750 ml
RNA Wash Buffer II Concentrate	40 ml	3 x 50 ml	2 x 200 ml
DNA Wash Buffer	20 ml	100 ml	200 ml
HB Buffer	55 ml	220 ml	650 ml
DEPC-ddH_2O	10 ml	40 ml	120 ml
Elution Buffer	25 ml	100 ml	250 ml
Instruction Manual	1	1	1

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

Important Notes

1. 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.

Before Starting

IMPORTANT	Dilute **RNA Wash Buffer II** Concentrate with **absolute ethanol** (96%-100%) before use: **R6732-00** Add 160 ml 96%-100% ethanol **R6732-01** Add 200 ml 96%-100% ethanol **R6732-02** Add 800 ml 96%-100% ethanol
	Dilute **DNA Wash Buffer** with **absolute ethanol** (96%-100%) before use **R6732-00** Add 80 ml 96%-100% ethanol **R6732-01** Add 400 ml 96%-100% ethanol **R6732-02** Add 800 ml 96%-100% ethanol

E-Z 96® DNA/RNA Protocol with Centrifugation

Materials supplied by user

- 96%-100% ethanol
- 70% ethanol
- β-Mercaptoethanol
- Multichannel pipet
- RNase-free filter pipette tips
- Reagent reservoirs for multichannel pipets
- Centrifuge with rotor for 96-well plates
- Disposable latex gloves
- 2ml 96-well deep well plate

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 by pipetting. Add 300 µl GTC Lysis Buffer directly to each well. Mix througly by pipetting up and down 10-20 times.

B. LYSIS OF SUSPENSION CULTURED CELLS: Transfer aliquots of up to 5 x 10^5 cells into the wells of a 96-well microplate. Spin the plate at 300 x g for 5 minutes. Remove the medium completely by pipetting. Add 300 µl GTC Lysis Buffer directly to each well. Mix throughly by pipetting up and down 10-20 times.

Note: *Add 20 µl ß-mercaptoethanol per 1ml GTC Lysis buffer before use. Complete removal of supernatant is critical for RNA isolation.*

2. Keep the microplate flat on the bench. Shake vigorously, end to end and side to side, for a total of one minute.

   Note: *If the multi-well plate used has volume less than 300 µl, reduce volume of the GTC lysis buffer to 200µl, as the total volume would be 400 µl after addition of 200µl ethanol in Step 6.*

3. Place the E-Z 96® DNA plate on top of the Square-Well Collection Plate and carefully add entire sample from Step 2 to each well of the E-Z 96® DNA plate.

4. Seal E-Z 96® DNA plate with sealing film. Load the E-Z 96® DNA/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 x g for 5 minutes at room temperature.

5. Store the E-Z 96® DNA plate at room temperature (15-25°C) or at 4°C for later DNA purification in steps 15-19. Use the flow-through for RNA purification in steps 6-14.

Total RNA Purification

6. Add one volume (300 µl) of 70% ethanol to the sample; mix throughly by pipetting up and down 3 to 4 times. Do no centrifuge.

   Note: *if some lysate was lost during previous DNA binding step, adjust the volume of ethanol accordingly.*

7. Place the E-Z 96® RNA plate atop the 2 ml deep-well plate and carefully transfer entire sample from Step 6 (including any precipitate) to each well of the E-Z 96® RNA plate.

8. Seal the E-Z 96® RNA plate with sealing film. Load the E-Z 96® 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 x g for 5 minutes at room temperature. Remove the sealing film and discard flow-through.

9. Add 500µl RNA Wash Buffer I directly into the each well of the E-Z 96® RNA plate, Seal the plate with new sealing film and centrifuge at 5,000 x g for 5 minutes at room temperature.

10. Remove the sealing film and add 700 µl Wash Buffer II diluted with ethanol to each well of the E-Z 96® RNA plate. Seal the plate with new sealing film. Centrifuge at 5,000 x 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*.*

11. Remove the sealing film. Add another 700 µl of RNA Wash Buffer II to each well of E-Z 96® RNA plate. **Do not seal the plate with film**. Centrifuge at 5,000 x g for 10 minutes at room temperature. The prolonged centrifugation is necessary to dry the E-Z 96® RNA plate.

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

12. Elution of RNA: Remove the sealing film and place the E-Z 96® RNA plate onto the microtube rack containing 1.2 ml microtubes (supplied with kit).

13. Add 50-75 µl DEPC-treated water to each well, and seal theE-Z 96® RNA plate with new sealing film(supplied with kit). Make sure to add water directly onto RNA matrix. Incubate for 3 minutes at room temperature. Centrifuge at 5,000 x g for 5 minutes at room temperature to elute RNA.
14. Remove the sealing film. Repeat Steps 12 and 13 for second elution.

    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*.

Genomic DNA Purification

15. Place the E-Z 96® DNA Plate on top of a 2ml Square-Well Collection Plate. Add 500µl HB Buffer to each well of E-Z 96® DNA Plate.

16. Seal the E-Z 96® RNA plate with sealing film. Load the E-Z 96® 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 x g for 5 minutes at room temperature. Remove the sealing film and discard flow-through. Re-use the collection plate for next step.

17. Add 800µl DNA Wash Buffer directly into the each well of the E-Z 96® DNA plate, **Do not seal the plate with film**. Centrifuge at 5,000 x g for 10 minutes at room temperature. The prolonged centrifugation is necessary to dry the E-Z 96® DNA plate.

18. Elution of DNA: Remove the sealing film and place the E-Z 96® DNA plate onto a microtube rack containing 1.2 ml microtubes (supplied with kit).

19. Add 100-200 µl Elution Buffer to each well, and seal theE-Z 96® DNA plate with new sealing film(supplied with kit). Make sure to add Elution Buffer directly onto DNA matrix. Incubate for 3 minutes at room temperature. Centrifuge at 5,000 x g for 5 minutes at room temperature to elute DNA

E-Z 96® DNA/RNA Vacuum Protocol

Materials supplied by user

- 96-100% ethanol
- 70% ethanol
- β-Mercaptoethanol
- 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 by pipetting. Add 300 ul GTC Lysis Buffer directly to each well.

   B. LYSIS OF SUSPENSION CULTURED CELLS: Transfer aliquots of up to 5 x 10^5 cells into the wells of a 96-well microplate. Spin the plate at 300 x g for 5 minutes. Remove the medium completely by pipetting. Add 300 ul GTC Lysis Buffer directly to each well. Mix by pipetting.

   Note: Add 20 µl ß-mercaptoethanol per 1ml GTC Lysis buffer before use. Complete removal of supernatant is critical for RNA isolation.

2. Keep the microplate flat on the bench. Shake vigorously, end to end and side to side, for a total of one minute.
   Note: *If the multi-well plate used has volume less than 300µl, reduce volume of the GTC lysis buffer to 200µl, as the total volume would be 400µl after addition of 200µl ethanol in Step 3.*

3. 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® DNA plate on the manifold's top section, making sure the E-Z 96® DNA plate is seated tightly on the rubber ring. Connect the vacuum manifold to the vacuum source. Keep the vacuum switch off.

4. Carefully transfer entire sample from Step 2 to each well of the E-Z 96® DNA plate. Seal the un-used wells with sealing film. Switch on the vacuum source. Apply vacuum until all the sample contents pass through the well membranes. Turn of the vacuum source and remove the E-Z 96® DNA plate.

   Note*: If some well of the E-Z 96® DNA plate is clogged, remove the E-Z 96® DNA plate and the Square-Well Collection Plate from manifold. Place E-Z 96® DNA plate on top of the Square-Well Collection Plate, centrifuge at 5000 x g for 5-10 minutes.*

5. Store the E-Z 96® DNA plate at room temperature (15-25°C) or at 4°C for later DNA purification in steps 17-19. Use the flow-through for RNA purification in steps 6-16.

Total RNA Purification

6. Add one volume (300µl) of 70% ethanol to the sample; mix throughly by pipetting up and down 3 to 4 times.

   Note: *If some lysate was lost during previous DNA binding step, adjust the volume of ethanol accordingly.*

7. Place a E-Z 96® RNA plate on top part of the vacuum manifold.

8. Carefully transfer entire sample from Step 6 to each well of the E-Z 96® RNA plate. Seal the un-used wells with sealing film. Switch on the vacuum source. Apply vacuum until all the sample contents pass through the well membranes.

9. Add 500 µl RNA Wash Buffer I directly into each well of the E-Z 96® RNA plate. Apply the vacuum until transfer is complete. Switch off the vacuum, and ventilate the manifold.

10. Add 800 µl RNA Wash Buffer II to each well of the of E-Z 96® 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.*

11. Add another 800 µl RNA Wash Buffer II to each well of the of E-Z 96® RNA plate and apply the vacuum until transfer is complete. Switch off the vacuum *and* ventilate the manifold.

12. Remove the E-Z 96® RNA plate from top plate of vacuum manifold, and strike the bottom of the E-Z 96® RNA plate on a stack of paper towels. Repeat for few times until no liquid is released onto the paper towels.

13. Place the E-Z 96® RNA plate back to the top plate of the manifold. Apply vacuum for 15 minutes. Turn off the vacuum and ventilate the manifold.

14. 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® RNA plate on top plate of manifold.

15. Elution RNA: Add 50-75 µl of DEPC-treated water to each well, and seal E-Z 96® 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 5-10 minutes. Switch off the vacuum and ventilate the manifold.

16. Repeat the elution with a second volume of 50-75 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.*

17. Remove the eluted RNA from vacuum manifold and store at -80°C.

Genomic DNA Purification

18. Reassemble the vacuum manifold. Place the E-Z 96® DNA plate from step 5 on top part of the vacuum manifold.

19. Add 500 µl HB Buffer directly into each well of the E-Z 96® DNA plate. Apply the vacuum until all liquid pass through the membrane. Switch off the vacuum.

20. Add 800 µl DNA Wash Buffer directly into each well of the E-Z 96® DNA plate. Apply the vacuum until all liquid pass through the membrane. Switch off the vacuum, and ventilate the manifold.

21. Remove the E-Z 96® DNA plate from top plate of vacuum manifold, and strike the bottom of the E-Z 96® DNA plate on a stack of paper towels. Repeat for few times until no liquid is released onto the paper towels.

22. Place the E-Z 96® DNA plate back to the top plate of the manifold. Apply vacuum for 15 minutes. Turn off the vacuum and ventilate the manifold.

23. Replace the Square-Well Collection Plate or microtube rack containing 1.2 ml microtubes (supplied with kit). Reassemble the manifold. Place the E-Z 96® DNA plate on top plate of manifold.

24. Elution DNA: Add 100-200µl of Elution Buffer to each well, and seal E-Z 96® DNA plate with new sealing film (supplied with kit). Make sure to add Elution Buffer directly onto DNA matrix. Incubate for 2 minute at room temperature. Switch on the vacuum source for 5-10 minutes. Switch off the vacuum and ventilate the manifold.

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

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:

Two 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 throughly 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 GTC Lysis Buffer	• Dissolve the carrier RNA with GTC Lysis Buffer and repeat the purification with new sample. • Repeat elution.
	RNA remains on the plate	• Pre-heat DEPC-water to 70° C prior to elution. • Incubate for 5 min with water prior to elution
	Plate is overloaded	• Reduce quantity of starting material.
Clogged well on DNA	Incomplete lysis	• Mix thoroughly after addition of GTC Lysis Buffer.. • Reduce amount of starting material
Degraded RNA	Source	• Do not freeze and thaw sample more than once. • Follow protocol closely, and work quickly.
	RNase contamination	• Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	• Ensure RNA Wash Buffer II has been diluted with 4 volumes of 100% ethanol as indicated on bottle. • 1 X Wash Buffer II must be stored at room temperature. • Repeat wash with Wash Buffer II.
	Inhibitors of PCR	• Use less starting material • Prolong incubation with Buffer GTC to completely lyse cells
DNA contamination	Too much starting material	• Reduce the cell number and repeat the extraction. • Perform DNase digestion
Little or no RNA eluted	Lost DNA during process	• Make sure DNA wash Buffer is diluted with absolute ethanol with correct amount of ethanol • use the pre-heated Elution Buffer (65°C) for DNA elution.
RNA contamination on genomic DNA elute		• Add 50µl of RNase A (20mg/ml) to the DNA wash Buffer.