E.Z.N.A.® MicroElute RNA Clean-up Kit

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Introduction and Overview

E.Z.N.A.® MicroElute RNA Clean-up Kit provides a rapid and easy method for the isolation and concentration of RNA from enzymatic reactions or for desalting RNA samples. Up to 50 µg or down to a picogram of RNA can be recovered with specially designed MicroElute RNA Mini Columns.

RNA purified using E.Z.N.A.® MicroElute RNA Clean-up Kits are ready for all downstream applications such as RT-PCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation.

The E.Z.N.A.® MicroElute RNA Clean-up Kits combine the reversible binding properties of HiBind® matrix, a new silica-based material with the speed of micro-column spin technology. A specially formulated high salt buffer system allows RNA molecules greater than 200 bases to bind to the matrix. RNA samples are first mixed with a lysis buffer that contains guanidine isothiocyanate followed by adding ethanol to create a binding condition. Samples are then applied to the MicroElute RNA Mini Columns to which total RNA binds, while contaminants are effectively washed away. High-quality RNA is finally eluted in Nuclease-free Water.

New in this edition:

November 2018:

- DEPC Water has been replaced with Nuclease-free Water. DEPC Water is no longer provided in this kit.
- PR032 (DEPC Water, 100 mL) has been discontinued and is no longer available to purchase.

Quantification and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm with a spectrophotometer. One OD unit measured at 260 nm corresponds to 40 µg/mL RNA. Nuclease-free Water is slightly acidic and can lower A_{260}/A_{280} ratios. Use TE buffer to dilute RNA prior to spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while an A_{260}/A_{280} ratio of 0.6 denotes pure protein. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Phenol has a maximum absorbance at 270 nm and can interfere with spectrophotometric analysis of DNA or RNA. Store RNA samples at -70°C in water. Under these conditions, RNA is stable for more than a year.

Integrity of RNA

It is highly recommended that RNA quality be determined prior to beginning all downstream applications. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis with ethidium bromide staining. The ribosomal RNA bands should appear as sharp, clear bands on the gel. The 28S band should appear to be double that of the 18S RNA band (23S and 16S if using bacteria). If the ribosomal RNA bands in any given lane are not sharp and appear to be smeared towards the smaller sized RNA, it is very likely that the RNA undergone degradation during the isolation, handling, or storage procedure. Although RNA molecules less than 200 bases in length do not efficiently bind to the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Kit Contents

Product	R6247-00	R6247-01	R6247-02
Purifications	5	50	200
MicroElute RNA Mini Columns	5	50	200
2 mL Collection Tubes	5	50	200
QVL Lysis Buffer	3 mL	40 mL	150 mL
RNA Wash Buffer II	2 mL	12 mL	50 mL
Nuclease-free Water	2 mL	30 mL	60 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® MicroElute RNA Clean-up Kit components are guaranteed for at least 24 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in QVL Lysis Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

- Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
R6247-00	8 mL
R6247-01	48 mL
R6247-02	200 mL

- Add 20 µL 2-mercaptoethanol (β-mercaptoethanol) per 1 mL QVL Lysis Buffer depending on the protocol. This mixture can be stored for 4 weeks at room temperature.

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.

- Equilibrate samples and reagents to room temperature before beginning this protocol. All steps should be carried out at room temperature unless otherwise noted. Work quickly, but carefully.

- Prepare all materials required before starting the procedure to minimize RNA degradation.

- Carefully apply the sample or solution to the center of the HiBind® RNA Mini Columns. Avoid touching the membrane with pipet tips.

- 2-mercaptoethanol is key in denaturing RNases and can be added to an aliquot of QVL Lysis Buffer before use. Add 20 µL 2-mercaptoethanol per 1 mL QVL Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

- The maximum starting sample should be limited to 50 µg or 200 µL due to the capacity of the MicroElute RNA Mini Column.

E.Z.N.A.® MicroElute RNA Clean-up Kit - Clean-up Protocol

This protocol is designed to recover RNA from enzymatic reactions. For RNA desalting or RNA concentration from sample using RNA-Solv® Reagent or other phenol-based reagents, please use the RNA Desalting Protocol on Page 9.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000 x *g*
- Vortexer
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- 2-mercaptoethanol
- 100% ethanol
- DEPC-treated water

Before Starting:

- Prepare RNA Wash Buffer II and QVL Lysis Buffer according to the "Preparing Reagents" section on Page 5.

1. Measure the volume of sample and adjust to 100 µL (for sample <100 µL) or 200 µL (for samples >100 µL).

   Note: If the starting sample is RNA pellet, dissolve the sample with DEPC-treated water.

2. Add 350 µL QVL Lysis Buffer (for 100 µL sample) or 700 µL QVL Lysis Buffer (for 200 µL sample). Vortex to mix thoroughly.

   Note: 2-mercaptoethanol should be added to QVL Lysis Buffer before use. Please see Page 5 for instructions.

3. Add 250 µL 100% ethanol (for 100 µL sample in Step 1) or 500 µL 100% ethanol (for 200 µL sample in Step 1). Vortex to mix thoroughly.

4. Insert a MicroElute RNA Mini Column into a 2 mL Collection Tube.

5. Vortex and transfer the entire sample from Step 3, including any precipitates that may have formed, to the HiBind® RNA Mini column. (The maximum capacity of the column is 700 µL. Larger volumes can be loaded successively).

6. Centrifuge at 10,000 x *g* for 15 seconds at room temperature.

7. Discard the filtrate and reuse the collection tube.

8. Add 500 µL RNA Wash Buffer II.

9. Centrifuge at 10,000 x *g* for 30 seconds.

   Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 5 for instructions.

10. Discard the filtrate and reuse the Collection Tube.

11. Add 500 µL RNA Wash Buffer II.

12. Centrifuge at 13,000 x *g* for 1 minutes.

13. Discard the filtrate and Collection Tube.

14. Transfer the HiBind® RNA Mini Column into a new 2 mL Collection Tube.

15. Centrifuge at maximum speed for 2 minutes to completely dry the HiBind® RNA Mini Column.

    Note: It is important to dry the HiBind® RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

16. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL or 2 mL microcentrifuge tube (not provided).

17. Add 15-30 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the HiBind® RNA Mini Column matrix.

18. Centrifuge at maximum speed for 1 minute and store eluted RNA at -70°C.

E.Z.N.A.® MicroElute RNA Clean-up Kit - Desalting Protocol

This protocol is designed to clean up and concentrate RNA from various sources such as RNA isolated with RNA-Solv® Reagent and other phenol-based reagents.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000 x *g*
- Vortexer
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- 2-mercaptoethanol
- 100% ethanol
- DEPC-treated water

Before Starting:

- Prepare RNA Wash Buffer II and QVL Lysis Buffer according to the "Preparing Reagents" section on Page 5.
- Heat Nuclease-free Water to 65°C.

1. Measure the volume of sample and adjust to 100 µL (for sample <100 µL) or 200 µL (for samples >100 µL).

   Note: If the starting sample is RNA pellet, dissolve the sample with DEPC-treated water.

2. Add 350 µL QVL Lysis Buffer (for 100 µL sample) or 700 µL QVL Lysis Buffer (for 200 µL sample). Vortex to mix thoroughly.

   Note: 2-mercaptoethanol should be added to QVL Lysis Buffer before use. Please see Page 5 for instructions.

3. Add 250 µL 100% ethanol (for 100 µL sample in Step 1) or 500 µL 100% ethanol (for 200 µL sample in Step 1). Vortex to mix thoroughly.

4. Insert a MicroElute RNA Mini Column into a 2 mL Collection Tube.

5. Vortex and transfer the entire sample from Step 3, including any precipitates that may have formed, to the HiBind® RNA Mini Column. (The maximum capacity of the column is 700 µL. Larger volumes can be loaded successively).

6. Centrifuge at 10,000 x *g* for 15 seconds at room temperature.

7. Discard the filtrate and reuse the collection tube.

8. Add 500 µL RNA Wash Buffer II.

9. Centrifuge at 10,000 x *g* for 15 seconds.

   Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 5 for instructions.

10. Discard the filtrate and reuse the Collection Tube.

11. Add 500 µL RNA Wash Buffer II.

12. Centrifuge at 13,000 x *g* for 1 minutes.

13. Discard the filtrate the Collection Tube.

14. Transfer the HiBind® RNA Mini Column into a new 2 mL Collection Tube.

15. Centrifuge at maximum speed for 2 minutes to completely dry the HiBind® RNA Mini Column.

    Note: It is important to dry the HiBind® RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

16. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL or 2 mL microcentrifuge tube (not provided).

17. Add 15-30 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the HiBind® RNA Mini Column matrix.

18. Centrifuge at maximum speed for 1 minute and store eluted RNA at -70°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **800-832-8896**.

Possible Problems and Suggestions

Problem	Cause	Solution
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Heat Nuclease-free Water to 70ºC prior to elution. • Incubate column for 10 minutes with water prior to centrifugation.
	Column is overloaded	• Reduce the quantity of the starting sample
Problem	Cause	Solution
Clogged column	Column is overloaded Lower centrifugation speed	• Completely homogenize the sample. • Increase centrifugation time. • Reduce amount of starting samples
Problem		Solution
Degraded RNA	RNase contamination	• Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem	Cause	Solution
Problems in downstream applications	Salt carry-over during elution	• Ensure RNA Wash Buffer II has been diluted with 100% ethanol as indicated on the bottle. • 1 X RNA Wash Buffer II must be stored and used at room temperature. • Repeat wash with RNA Wash Buffer II
DNA contamination		• Digest with RNase-free DNase and inactivate at 75ºC for 5 min.
Low Abs ratios	RNA diluted in acidic buffer or water	• Nuclease-free Water is slightly acidic and can lower A_{260}/A_{280} ratios. Use TE buffer to dilute RNA prior to spectrophotometric analysis.