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Introduction

E.Z.N.A.® MicroElute RNA Clean-up Kit provides a rapid and easy method for the purification and concentrate RNA from enzymatic reactions or for desalting the RNA samples. Up to 50 µg or down to picogram of RNA can be recovered with specially designed MicroElute RNA column. RNA purified using E.Z.N.A.® MicroElute RNA Clean-up Kit is ready for all downstream applications such as RT-PCR*, Northern blotting, poly A^+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation.

Principle

The E.Z.N.A.® MicroElute RNA Clean-up Kit combines 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 lysis buffer contains guanidine isothiocyanate followed by adding ethanol to create binding condition. Samples are then applied to the HiBind® MicroElute columns to which total RNA binds, while contaminants are effectively washed away. High quality RNA is finally eluted in DEPC-treated sterile water.

Storage and stability

E.Z.N.A.® MicroElute RNA Clean-up Kit should be stored at room temperature. During shipment crystals may form in the TRK Lysis Buffer. Warm to 37°C to dissolve. 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 22-25°C.

Binding Capacity

Each HiBind™ MicroElute Column can bind up to 50 ug of total RNA. Use more than 50ug of total RNA is not recommended.

*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

Product Number	R6247-00	R6247-01	R6247-02
Purification Times	5 Preps	50 Preps	200 Preps
HiBind® MicroElute RNA Columns	5	50	200
2 ml Collection Tubes	10	100	400
QVL Lysis Buffer	5 ml	30 ml	100 ml
RNA Wash Buffer II Concentrate	5 ml	12 ml	3 x 12 ml
Linear Acrylamide	30 µl	250 µl	1mL
DEPC-ddH_2O	-	5 ml	20 ml
Instruction Manual	1	1	1

Before Starting

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

IMPORTANT	**RNA Wash Buffer II Concentrate** must be diluted with absolute ethanol before. R6247-00 Add 20 ml 96-100% ethanol R6247-01 Add 48 ml 96-100% ethanol R6247-02 Add 48 ml 96-100% ethanol to each bottle **Store diluted RNA Wash Buffer II at room temperature**

- 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.
- During the procedure work carefully but quickly.
- Maxium starting sample should be limited to 50µg or 200µl due to the capacity of MicroElute RNA column.
- Under cool ambient conditions, crystals may form in QVL Lysis Buffer. This is normal and the bottle should be warmed to re-dissolve the salt.
- All centrifugation steps must be carried out at 22°C-25°C.

E.Z.N.Z.™ MicroElute RNA Clean-up Protocol

This protocol is designed to recovery RNA from enzymatic reactions such as DNase I digestion, In vitro transcription, etc. For RNA desalting or clean-up from sample using RNA-Solv Reagent or other phenol involved reagents, please use RNA desalting protocol on page 5.

1. **Measure the volume of sample and adjust the sample volume to 100µl with DEPC-Water and proceed to step 2.**

2. **Add 350ul QVL Lysis buffer and mix by vortexing at maximum speed for 15 seconds.** When process small amount of RNA (≤2 µg). Add 2 µl of Linear Acrylamide to the mixture.
   Note: Remember to add 20 µl of 2-mercaptoethanol per 1 ml of QVL Lysis Buffer before use.

3. **Add 250ul absolute ethanol (96-100%, room temperature) to the sample** and mix thoroughly by vortexing.

4. **Apply sample from step 3 to HiBind® MicroElute RNA column inserted in a 2 ml collection tube (supplied).** The maximum capacity of the spin cartridge is 700 µl. (Larger volumes can be loaded successively.) A precipitate may form upon addition of ethanol in Step 3. Vortex and add the entire mixture to the column. With the spin column inside a 2ml collection tube (supplied with kit), centrifuge at 10,000 x g for 30 seconds at room temperature. Discard flow-through and re-use the collection tube.

5. **Place column in the same 2 ml collection tube from step 4 and add 500 µl RNA Wash Buffer II diluted with absolute ethanol**. Centrifuge and discard flow-through.

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

6. **Place column back into the same collection tube from step 5, and add another 500 µl RNA Wash Buffer II diluted with absolute ethanol.** Centrifuge and discard the flow-through and collection tube.

7. **Place the column into a new 2 ml collection tube (supplied). Centrifuge the column at full speed (≥13,000 x g) for 2 minutes.** Discard the flow-through and the collection tube.

8. **Elution of RNA. Transfer the column to a clean 1.5 ml microfuge tube (not supplied with kit) and pipet 15-30 µl of DEPC-treated water (supplied with kit) into the column.** Make sure to add water directly onto center of column matrix. Incubate at room temperature for 2 min. Centrifuge for 1 min at maximum speed. A second elution may be necessary if the expected yield of RNA >20 µg.

   Alternatively, RNA may be eluted with a smaller volume of water. While reduced elution volume reduce the RNA yield, the concentration will be higher since more than 80% of RNA is recovered with the first elution. Pre-heating the water to 65°C before adding to column and incubating column 2 min at room temperature before centrifugation may increase yields. Elution with less than 10µl volume is not recommended.

E.Z.N.Z.™ MicroElute RNA Desalting and Concentration 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 involved reagents.

1. **Measure the volume of sample and transfer into a new 1.5 ml microcentrifuge tube.** Adjust the sample volume to 100µl with DEPC-Water and proceed to step 2.

   Note: if the starting samples is RNA pellet, dissolve the samples with DEPC treated water.

2. **Add 350ul QVL Lysis buffer and mix by vortexing.** When process small amount of RNA (≤2 µg). Add 2 µl of Linear Acrylamide to the mixture.

   Note: Remember to add 20 µl of 2-mercaptoethanol per 1 ml of QVL Lysis Buffer before use.

3. **Add 250 ul absolute ethanol (96-100%, room temperature) to the sample and mix thoroughly by vortexing.**

4. **Apply sample from step 3 to HiBind® MicroElute RNA column inserted in a 2 ml collection tube (supplied).** The maximum capacity of the spin cartridge is 700 µl. (Larger volumes can be loaded successively.) A precipitate may form

upon addition of ethanol in Step 3. Vortex and add the entire mixture to the column. With the spin column inside a 2ml collection tube (supplied with kit), centrifuge at 10,000 x g for 30 seconds at room temperature. Discard flow-through and re-use the collection tube.

5. **Place column in the same 2 ml collection tube from step 4 and add 500 µl RNA Wash Buffer II diluted with absolute ethanol.** Centrifuge and discard flow-through.

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

6. **Place column back into the same collection tube from step 5, and add another 500 µl RNA Wash Buffer II diluted with absolute ethanol.** Centrifuge and discard the flow-through and collection tube.

7. Place the column into a new 2 ml collection tube (supplied). Centrifuge the column at full speed (≥13,000 x g) for 2 minutes. Discard the flow-through and the collection tube.

8. **Elution of RNA. Transfer the column to a clean 1.5 ml microfuge tube (not supplied with kit) and pipet 15-30 µl of DEPC-treated water (supplied with kit) into the column.** Make sure to add water directly onto center of column matrix. Incubate at room temperature for 2 min. Centrifuge for 1 min at maximum speed. A second elution may be necessary if the expected yield of RNA >20 µg.

   Alternatively, RNA may be eluted with a smaller volume of water. While reduced elution volume reduce the RNA yield, the concentration will be higher since more than 80% of RNA is recovered with the first elution. Pre-heating the water to 65°C before adding to column and incubating column 2 min at room temperature before centrifugation may increase yields. Elution with less than 10µl volume is not recommended.

Quality of RNA

It is highly recommended that RNA quality be determined prior to all analyses. The quality of RNA can best be assessed by denaturing agarose gel electrophoresis and ethidium bromide staining. Two sharp bands should appear on the gel for total cellular RNA. These are the 28S and 18S (23S and 16S for bacteria) ribosomal RNA bands. If these band smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling, or storage. Although RNA molecules less than 200 bases in length do not efficiently bind the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Quantification of RNA

The quantity of the RNA can be determined by measure the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$RNA\ concentration = Absorbance_{260} \times 44 \times (Dilution\ Factor)\ \mu g/ml$$

This calculation is only valid when the pH of the RNA solution is neutral (pH 7.0). The ratio of ($absorbance_{260}$)/($absorbance_{280}$) is an indication of nucleic acid purity. A value greater than 1.9 indicates good quality of RNA. Alternatively, quantity (as well as quality) can sometimes best be determined by Agilent 2100 Bioanalyser by comparison the ration of 28S and 18S RNA.

Troubleshooting Guide

Problem	Cause	Suggestion
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Pre-heat DEPC-water to 65°C prior to elution. • Incubate column for 10 min with water prior to centrifugation.
	Column is overloaded	• Reduce quantity of starting sample
Clogged column	Column is overloaded Lower centrifugation speed	• Completely homogenize sample. • Increase centrifugation time. • Reduce amount of starting samples
Degraded RNA	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 Concentrate has been diluted with 4 volumes of 100% ethanol as indicated on bottle. • 1 x RNA Wash Buffer II must be stored and used at room temperature. • Repeat wash with Wash Buffer II.
DNA contamination		• Digest with RNase-free DNase and inactivate at 75°C for 5 min.
Low Abs ratios	RNA diluted in acidic buffer or water	• DEPC-treated water is acidic and can dramatically lower Abs260 values. Use TE buffer to dilute RNA prior to spectrophotometric analysis.