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Introduction

E.Z.N.A.™ MicroElute RNA Clean-up Kit provides a rapid and easy method for the isolation 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 Kits 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 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 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

E.Z.N.A.™ MicroElute RNA Clean-up Kits should be stored at room temperature. During shipment crystals may form in the QVL 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.

*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

MicroElute RNA Clean-up Kit	R6247-00	R6247-01	R6247-02
HiBind® MicroElute RNA Columns	5	50	200
2 mL Collection Tubes	10	100	400
QVL Lysis Buffer	3 mL	40 mL	150 mL
RWB Buffer	2.5 mL	12 mL	50 mL
DEPC-ddH_2O	1 mL	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	Dilute **RWB Buffer** with **absolute ethanol** before use **R6247-00** Add 10mL 100 % ethanol **R6247-01** Add 48mL 100% ethanol **R6247-02** Add 200mL 100% ethanol

- 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 limitted 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 redissolve the salt.
- 2-mercaptoethanol (ß-mercaptoethanol) is key in denaturing RNases and must be added to an aliquot of QVL Lysis Buffer before use. Add 20 µl of 2-mercaptoethanol per 1 mL of QVL Lysis Buffer. This mixture can be stored for 1 week at room temperature.
- All centrifugation steps must be carried out at 22°C-25°C.

Equipment and reagents supplied by user

- Absolute ethanol (96-100%)
- Micro-centrifuge capable of 20,000 x g
- RNase-free microcentrifuge tubes and tips
- Disposable latex gloves
- DEPC-treated water

RNA Clean-up Protocol

This protocol is designed to recovery DNA from enzymatic reactions. 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 (for sample less than 100µl) or 200µl (for sample large than 100µl) and proceed to Step 2.

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

2. **Add 350µl (for 100µl sample)or 700 µl (for 200µl sample)of QVL Lysis buffer and mix by vortexing.**

3. Add 250 µl (**(for 100µl sample in step 1)** or 500 µl (**(for 200µl sample in step1)** absolute ethanol (96-100%) to the sample and mix thoroughly by vortexing.

4. Apply sample to HiBind® RNA MicroElute 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 15 seconds **at room temperature**. Discard flow-through and re-use the collection tube.

5. **Place column in the same 2mL collection tube** from step 4 and add 500 µl RWB Buffer. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through and reuse the collection tube in next step.

   Note: RWB must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

6. Add 500 µl RWB to the column. Centrifuge at 13000 x g for 2 minutes to dry the membrane. Discard flow-through and collection tube.

   Note: Carefully remove the spin column from the collection tube so the column does not contact with the flow-through.

7. **Transfer the column into a new collection tube (supplied)**. Open the cap of the column, centrifuge at maximum speed for 5 minutes. Discard the flow-through and the collection tube.

   Note: It is critical to completely dry the membrane before the elution since the residue ethanol from RWB Buffer could interfere the downstream applications. Centrifuging with open cap ensures that not ethanol is carried over during elution step.

8. **Elution of RNA.** Transfer the column to a clean 1.5 mL microfuge tube (not supplied with kit) and pipet 15µl of DEPC-treated water (supplied with kit) into the column. Make sure to add water directly onto center of column matrix. Centrifuge 1 min at maximum speed.

RNA 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 involved reagents.

Additional materials required by user

- Absolute ethanol (96-100%)
- Micro-centrifuge capable of 20,000 x g
- RNase-free microcentrifuge tubes and tips
- Disposable latex gloves
- DEPC-treated water

Measure the volume of sample and adjust the sample volume to 100µl (for sample less than 100µl) or 200µl (for sample large than 100µl) and proceed to Step 2.

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

1. **Add 350µl (for 100µl sample)or 700 µl (for 200µl sample)of QVL Lysis buffer and mix by vortexing. .**

2. Add 250 µl (**(for 100µl sample in step 1)**or 500 µl (**(for 200µl sample in step 1)**100% absolute ethanol to the sample and mix thoroughly by vortexing.

3. **Apply sample to HiBind® RNA MicroElute 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 15 seconds at room temperature. Discard flow-through and the collection tube.

4. **Place column in a clean 2mL collection tube (supplied)**, and add 500 µl RWB Buffer. Centrifuge at 10, 000 x g for 15 seconds. Discard flow-through and re-use the collection tube.

5. **Place column in the same 2mL collection tube** from step 4 and add 500 µl RWB Buffer. Centrifuge at 13,000 x g for 2 minutes. Discard flow-through and collection tube.

   Note: Carefully remove the spin column from the collection tube so the column does not contact with the flow-through.

6. **Transfer the column into a new collection tube (supplied)**. Open the cap of the column, centrifuge at maximum speed for 5 minutes. Discard the flow-through and the collection tube.

   Note: It is critical to completely dry the membrane before the elution since the residue ethanol from RWB Buffer could interfere the downstream applications. Centrifuging with open cap ensures that not ethanol is carried over during elution step.

7. **Elution of RNA.** Transfer the column to a clean 1.5 mL microfuge tube (not supplied with kit) and pipet 15µl of DEPC-treated water (supplied with kit) into the column. Make sure to add water directly onto center of column matrix. Centrifuge 1 min at maximum speed.

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.

Quantitation 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 Tips

Problem	Cause	Suggestion
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Pre-heat DEPC-water to 70° 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 RWB Buffer has been diluted with 2.5 volumes of 100% ethanol as indicated on bottle. • 1 X RWB Buffer must be stored and used at room temperature. • Repeat wash with RWB Buffer
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.