E.Z.N.A.® RNA Probe Purification Kit

Product R6248 & R6249

Introduction

E.Z.N.A.® RNA Probe Purification Kit allows rapid and convenient recovery of up to 50 µg RNA from any transcription reaction. HiBind® spin cartridges and optimized buffers facilitate isolation of pure RNA from nucleotides, unincorporated label, enzymes, and salts. The protocol is simple and fast ensuring minimal RNase contamination and consistent results. Both radioisotopic and non-radioactive probes may be purified with the system.

Benefits
The E.Z.N.A.® RNA Probe Purification is:

- **Fast** - less than 20 minutes to recover pure RNA from a standard transcription reaction
- **Safe** - no phenol/chloroform extractions
- **Convenient** - allowing multiple samples to be processed in parallel.
- **Reliable** - giving consistent yields at high quality

New in this edition
- **New designed V-Spin column introduced (Kit # R6249)**
- **Capped V-spin column with vacuum luer made it possible for vacuum process.**

Kit Contents

Product Number	**R6248-00** **R6249-00**	**R6248-01** **R6249-01**	**R6248-02** **R6249-02**
HiBind® RNA columns	5	50	200
2 ml collecting tubes	10	100	400
Buffer RB	2 ml	30 ml	120 ml
RWB Buffer	5 ml	12 ml	50 ml
DEPC-treated sterile water	1 ml	5 ml	20 ml
Instruction Manual	1	1	1

Storage and Stability

All components of the RNA Probe Purification Kit are stable for at least 24 months from the date of purchase when stored at 22°C-25°C.

Material to Be Provided by User

- Absolute (96%-100%) ethanol
- 2-mercaptoethanol (ß-mercaptoethanol)
- Sterile 1.5 ml microfuge tubes
- Tabletop microcentrifuge capable of 10,000 x g

Before Starting

IMPORTANT	RWB Buffer must be diluted with absolute ethanol before use: **R6248-00 & R6249-00**: Add 20 ml absolute (96%-100%) ethanol to bottle. **R6248-01& R6249-01**: Add 48 ml absolute (96%-100%) ethanol to bottle. **R6248-02& R6249-02**: Add 200 ml absolute (96%-100%) ethanol to bottle.

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.

- 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.

- Under cool ambient conditions, crystals may form in Buffer RB. 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 Buffer RB before use. Add 20 µl of 2-mercaptoethanol per 1 ml of Buffer RB. This mixture can be stored for 1 week at room temperature.

- All centrifugation steps must be carried out at 22°C-25°C.

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RNA Probe Purification Procedure

1. Adjust RNA sample volume to 100 µl with DEPC-treated H_2O (provided). For larger volumes adjust all other buffers in proportion.

2. Add 350 µl Buffer RB/2-mercaptoethanol and mix thoroughly.

   For radioisotopic labeling reactions add 2-5 µg carrier RNA (tRNA or rRNA) per ml of Buffer RB/2-mercaptoethanol before use. This mixture is stable at -70oC for 2-3 months but will require brief heating/vortexing to redissolve salts. Non-radioactive labeling reactions generally do not require carrier since yields exceed 1 µg RNA.

   NOTE: Add 20 **µ**l of 2-mercaptoethanol per 1 ml of Buffer RB before use. This mixture may be stored at Room temperature for 1 week.

3. Add 250 µl absolute ethanol to the sample and mix. A precipitate may form on addition of ethanol. This will not interfere with RNA purification.

4. Apply entire sample from previous step onto HiBind® RNA spin column assembled in a 2 ml collecting tube (supplied). Centrifuge 20 seconds at 10,000 x g (approx 12,500 rpm on most microcentrifuges).

5. Discard flow-through liquid from step 4. Wash the column by adding 700 µl RWB Buffer. Centrifuge as above.

   NOTE: RWB Buffer must be diluted with absolute ethanol as described on page 1.

6. Discard liquid and place column into a **NEW** 2ml collecting tube. Pipet 500 µl RWB Buffer onto spin column. Centrifuge as above. Discard flow-through and re-use the collection tube.

7. Place the column into the same collection tube from previous step. Spin empty column 2 min at 10,000 x g to dry HiBind® matrix. This is critical for removing residual ethanol which may otherwise be carried over during elution and interfere with downstream applications.

9. Transfer spin column to a new 1.5 ml microfuge tube. Elute the RNA by pipetting 30-50 µl DEPC-treated water (supplied) directly onto matrix. Centrifuge 1 minute at 8,000 x g (approx 10,000 rpm on most microcentrifuges).

10. The first elution represents 80%-85% of bound RNA. A second elution will yield an additional 10%-15%. For yields >30 **µ**g RNA, repeat elution, use a larger volume of water, or use the first eluate to elute a second time. Preheating the water to 80°C prior to elution and incubating the soaked column at room temperature for 2 minutes before centrifugation may also improve the yield.

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Troubleshooting Guide

Problem	Cause	Suggestion
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Pre-heat DEPC-water to 80° C prior to elution. • Incubate column for 2 min with water prior to centrifugation.
	Column is overloaded	• Reduce quantity of starting material.
	Too little starting material used.	• Increase starting material to at least 10 µg.
Degraded RNA	Source	• Freeze starting material quickly in liquid nitrogen. • 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 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.
Low O.D. 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. • Also sub-microgram quantities of RNA are usually not quatifiable spectrophotometrically. A fluorometric determination may be necessary.

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