MediRes Corp.

USER GUIDE **G3640-50T**

RNA Extraction Kit (Spin Column Based)

Product #: G3640-50T
Quantity: 50 reactions

Product Description

- Designed for rapid and cost-effectives purification of RNA from cultured cells (adherent and non-adherent) and animal tissues.
- High purity RNA extraction is achieved by combining proprietary silica-based membrane technology in the form of a convenient spin column. The kit utilizes two columns: **gDNA Eraser Spin Column** (to remove genomic DNA) and an **RNA Spin Column** (to bind RNA).
- The procedure avoids phenol and chloroform. The purified RNA contains no protein and no genomic DNA contamination.
- The high-quality RNA can be used in Northern blotting, dot blotting, mRNA purification, *in vitro* translation, RNase protection assays, RT-PCR, Real Time RT-PCR, cDNA library construction and other molecular experiments including direct sequencing.

Kit Components – BAG #1 (store at room temperature)

Buffer RL1	30 mL
Buffer RW1	12 mL (*note: add 18 mL 100% ethanol per bottle before use*)
Buffer RW2	20 mL (*note: add 80 mL 100% ethanol per bottle before use*)
Nuclease-free Water	12 mL
gDNA Eraser Spin Column	50 columns
RNA Spin Column	50 columns
10×DNase Buffer	500 µL

Kit Components – BAG #2 (store at -20^{0}C)

DNase	250 µL
DTT Solution	1.2 mL

Additional Required Reagents

100% Ethanol
Isopropanol

Important Preparatory Notes (PLEASE READ CAREFULLY)

1. If precipitate forms in **Buffer RL1** during storage, solution should be re-dissolved by incubating at 37℃, then cooled back down to 25℃ before use.
2. Add **18 mL 100% ethanol** to the **Buffer RW1** and mark the bottle on the label.
3. Add **80 mL 100% ethanol** to the **Buffer RW2** and mark the bottle on the label.
4. Add appropriate volume of **DTT Solution** into **Buffer RL1** to a final concentration of 4% (i.e. 40 µL DTT Solution per 1 mL of Buffer RL1). This addition should be prepared immediately before use. This mixture can be stored at 4℃ for one month.
5. Always keep buffer bottles tightly closed and keep columns sealed in the enclosed zip-lock bag.
6. Proper laboratory safety practices should be employed, including the use of lab coats, gloves, and eye protection.

RNA Extraction Protocol

1. **Sample preparation:**

   - **Non-adherent suspension cells**: Transfer cells to an appropriately sized RNase-free tube and centrifuge at 1,000 rpm for 5 minutes at 4°C to pellet. Discard the growth medium from the tube. Add **500 µL** of **Buffer RL1** prepared with **DTT solution** to the sample. Pipet up and down until no significant pellet. Let the sample stand at room temperature for 2 minutes.

   - **Adherent cells:** Remove the growth medium from the cells and wash cells with 1×PBS buffer (pH 7.4). Add **500 µL** of **Buffer RL1** prepared with **DTT solution** evenly over the cell monolayer. Pipet the cells up and down several times. Transfer the lysate to a 1.5 ml RNase-free tube. Let the sample stand at room temperature for 2 minutes.

   - **Animal tissue:** Transfer 2-20 mg of fresh or cryopreserved animal tissue to an appropriately sized RNase-free tube. Add two or three 4 mm stainless

steel grinding beads (Cat# G0104) per sample. Immediately add **500 µL** of **Buffer RL1** prepared with **DTT solution** to the sample. Use an automated homogenizer (TissueLyser) to homogenize thoroughly (2 to 5 min @ 20-50 Hz). Let the sample stand at room temperature for 2 minutes.

2. Transfer sample to **gDNA Eraser Spin Column**. Centrifuge at 12,000 rpm at 4°C for 1 minute.
3. Discard the **gDNA Eraser Spin Column** while keeping the flow-through in the collection tube.
4. Add **2/3 volume** of **isopropanol** (*note: visible precipitate may form after adding isopropanol*) to the flow-through in the collection tube. Pipet up and down to mix well.
5. Transfer up to 600 µL of the sample (including any remaining precipitate) to the **RNA Spin Column**.
6. Centrifuge at 12,000 rpm for 30 seconds at room temperature. Discard the flow-through, and reinsert the **RNA Spin Column** in the same collection tube.
7. Add **500 µL Buffer RW1** to the spin cartridge. Centrifuge at 12,000 rpm for 30 seconds at room temperature, and discard the flow-through. Reinsert the **RNA Spin Column** in the same collection tube.
8. Add **600 µL Buffer RW2** to the spin cartridge (*note: add Buffer RW2 along the wall of the RNA Spin Column to help flush the residual salt*). Centrifuge at 12,000 rpm for 30 seconds at room temperature, and discard the flow-through. Reinsert the **RNA Spin Column** in the same collection tube.
9. Optional DNase digestion: The **gDNA Eraser Spin Column** can effectively remove most of the genomic DNA. For some tissues with high DNA content (such as liver, kidney, spleen), perform the optional DNase digestion steps:
    - Add **5 µL DNase** to **5 µL 10X DNase Buffer** and **40 µL RNase-free Water** for a total of 50 µL in a new 1.5 mL RNase-free centrifuge tube. Mix by gently pipetting up and down several times.
    - Pipet all **50 µL** of the **DNase mixture** onto the center of the **RNA Spin Column** membrane. Incubate at room temperature for 15 minutes.

10. Add **500 µL Buffer RW2** to the spin cartridge. Centrifuge at 12,000 rpm for 30 seconds at room temperature, and discard the flow-through. Reinsert the **RNA Spin Column** in the same collection tube.
11. Repeat Step 10.
12. Centrifuge the **RNA Spin Column** at 12,000 rpm for 2 minutes to dry the membrane with RNA.
13. Discard the flow-through and the Collection Tube and insert the **RNA Spin Column** into a **new 1.5 mL RNase-free centrifuge tube**. Incubate at room temperature for 3-5 minutes to completely evaporate residual ethanol in the RNA Spin Column.
14. Add **50-100 µL Nuclease-Free Water** to the center of the **RNA Spin Column**. Incubate at room temperature for 5 minutes.
15. Centrifuge the **RNA Spin Column** for 2 minutes at 12,000 rpm at room temperature to elute the RNA from the membrane into the RNase-free centrifuge tube.
    - If more yield is required, the elution can be re-applied into the center of the membrane. Incubate for 5 minutes at room temperature and centrifuge at 12,000 rpm for 2 minutes to elute the RNA.
16. Store the purified RNA at -80℃ for long-term storage. Avoid repeated freezing and thawing of the DNA.

Approximate RNA Yield:

Sample species	Sample names	RNA yield
Animal tissues	Mouse liver	30-45 μg/10 mg
	Mouse spleen	20-30 μg/10 mg
	Mouse lung	10-20 μg/10 mg
	Mouse kidney	20-30 μg/10 mg
	Mouse heart	5-10 μg/10 mg
	Mouse thymus	10-20 μg/10 mg
	Mouse brain	5-10 μg/10 mg
	Mouse pancreas	5-15 μg/10 mg
Cells	Hela	8-15 μg/10^6 cells

NOTES:

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