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Ribology Total RNA Isolation Kit

(For isolation of RNA from animal cells and animal tissues)

For Research Use Only

v202505

Kit Contents

Cat. No. / preps	RiboStart (10 preps)	RiboMini (50 preps)
RiboLyse	20 ml	40 ml
RiboWash I	25 ml	35 ml
RiboWash II (Concentrate)	10 ml	15 ml
RiboElute (RNase-free Water)	5 ml	8 ml
RiboSpin Column	10 pcs	50 pcs
Collection Tube (2 ml)	50 pcs	200 pcs
Elution Tube (1.5 ml)	4 pcs	50 pcs

Add RNase-free ethanol (96–100%) to RiboWash II before first use.

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Specification

- Principle: micro spin column (silica membrane)
- Operation time: ~30 min
- Binding capacity: ≤100 µg RNA per column
- Column compatibility: centrifugation
- Minimum elution volume: 20 µl

Sample Amount

Sample Type	Starting Material	RiboLyse Volume (µl)
Animal Cells	<5 × 10^6 cells	100–300
	<1 × 10^7 cells	600
Animal Tissues	<20 mg	300
	≤30 mg	600

Important Notes

- Ensure all plastics (tubes, tips, columns) are RNase-free.
- Buffers contain irritants — wear gloves and a lab coat.
- β-Mercaptoethanol is hazardous — use in a fume hood.
- Add ethanol to RiboWash II before first use.
- All centrifugation is performed at full speed (~14,000 × g).
- Optional: 2-mercaptoethanol (β-ME) may be added to Ribolyse at a 1:100 dilution to enhance RNA protection.

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General Protocol: Animal Cells

1. For adherent cells

- Wash the culture vessel once with PBS, aspirate completely, and add the appropriate volume of RiboLyse directly to the well or flask.
- Allow RiboLyse to sit for ~1 minute or gently swirl to aid lysis.

2. For suspension cells

- Collect cells, centrifuge briefly, discard supernatant, wash once with PBS, and add the correct volume of RiboLyse to the pellet.
- Pipette up/down to ensure complete lysis.

3. Clarify lysate if needed

- If visible debris is present, centrifuge lysate at maximum speed for 3 minutes.
- If no debris is visible, skip this step and proceed directly to ethanol addition (step 5).

4. Transfer clarified lysate

- Move the clear supernatant to a fresh RNase-free microcentrifuge tube.

5. Add ethanol

- Add 1 volume of 70% ethanol to the lysate and mix thoroughly:
  - Pipette up and down ~5 times or
  - Gently vortex for ~5 seconds.
  - This creates optimal binding conditions for RNA.

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6. Bind RNA to RiboSpin column

- Load up to 600 µl of the lysate/ethanol mixture onto a RiboSpin Column placed in a 2 ml collection tube. Centrifuge 60 s at 14,000 × g. Repeat step 6 until all lysate/ethanol mixture is processed.

Wash Steps

7. RiboWash I

- Add 500 µl RiboWash I, centrifuge 60 s at 14,000 × g.
- Discard flow-through and place the column into a fresh 2 ml collection tube.

8. RiboWash II (1st wash)

- Add 600 µl RiboWash II, centrifuge 60 s at 14,000 × g.
- Discard flow-through and move the column to a new collection tube.

Note: Ensure ethanol has been added to RiboWash II before use.

9. RiboWash II (2nd wash)

- Add 600 µl RiboWash II, centrifuge 2 min at 14,000 × g.
- Discard flow-through and place the column into a fresh tube.

10. Dry spin

- Centrifuge the empty column 60 s at full speed to remove residual ethanol.
- Important: Incomplete drying may inhibit downstream enzymatic reactions.

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Elution

11. Prepare column for elution

- Place the RiboSpin Column into a clean **1.5 ml Elution Tube**.

12. Add RiboElute

- Add the desired volume of **RiboElute (RNase-free water)** to the center of the membrane.
- Let the column stand 1 minute to maximise elution efficiency.

13. Elute RNA

- Centrifuge **60 s at 14,000 × g** to collect purified RNA.

14. Storage

- Store RNA at –70°C for long-term preservation. *Optional pre-protocol: Animal tissue homogenization & lysate clarification (≤30 mg tissue)*

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Animal Tissue

Before you start

- Tissue input: up to 30 mg per sample.
- Keep RNases down: work quickly, keep tissue cold, use RNase-free plastics, change gloves often.

Prepare tissue

- Excise tissue (≤30 mg) and place immediately into RNase-free tube.
- Optional (recommended): briefly blot excess liquid (blood/PBS) on clean tissue or gauze.
- If not processing immediately: snap-freeze and store at −80 °C.

Homogenize tissue in RiboLyse

- Add RiboLyse directly to the tissue, then homogenize using one of the methods below.

Method A — Bead beating (fast + consistent; best for most tissues)

Best for fibrous tissues (muscle), spleen, small biopsies, mixed tissue types

- Add tissue to a tube containing RNase-free beads (stainless steel or ceramic).
- Add RiboLyse and secure the tube in a bead mill.
- Homogenize in short bursts until no visible pieces remain.
- Briefly spin down to collect liquid from the cap.

Tip: Avoid overheating—use short runs with a brief pause if needed.

Method B — Rotor-stator homogenizer

- Add tissue + RiboLyse to a suitable tube (deep enough to prevent splashing).
- Homogenize until the lysate looks uniform (no chunks).
- Rinse the probe in RNase decontamination solution between samples to prevent carryover.

Method C — Mortar and Pestle

- Chill mortar/pestle (and ideally use liquid nitrogen).
- Grind frozen tissue to a fine powder.
- Transfer powder to an RNase-free tube and immediately add RiboLyse.
- Mix thoroughly by vortexing or pipetting until uniform.

GO TO STEP 3 IN PROTOCOL (Animals Cells) Clarify lysate (remove insoluble debris)

3. Clarify lysate if needed

- If visible debris is present, centrifuge lysate at maximum speed for 3 minutes.

4. Transfer clarified lysate

- Move the clear supernatant to a fresh RNase-free microcentrifuge tube.

PROCEED WITH STEP 5 IN PROTOCOL (Animal cells)