Standard Operating Procedure

Protocol Title	Spin-Column Isolation of Total RNA from Cultured Mammalian Cells
Protocol ID / Version	SOP-BIO-004 / Version 2.1
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Effective Date	October 12, 2026
Approved By	Dr. Marcus Vance, Director of Quality Assurance

1 Purpose & Scope

This protocol outlines the standard operating procedure for the spin-column isolation of high-quality total ribonucleic acid (RNA) from cultured mammalian cell lines (including suspension cells and adherent monolayers). It covers the extraction process for up to 10^7 cells per prep. The primary objective is to yield intact, DNA-free total RNA suitable for sensitive downstream applications such as reverse-transcription quantitative PCR (RT-qPCR), high-throughput RNA sequencing (RNA-seq), and microarray hybridization.

2 Safety & PPE Requirements

⚠ SAFETY WARNINGS & PPE REQUIREMENTS

- **Chemical Hazards:** Lysis buffers contain guanidinium isothiocyanate. Contact with acids or bleach releases toxic hydrogen cyanide gas. Never expose guanidinium waste to bleach.
- **Toxic Additives:** Perform all handling and addition of β-mercaptoethanol (β-ME) to lysis buffers in a certified chemical fume hood due to its high volatility and toxicity.
- **PPE:** Standard laboratory attire is mandatory. Double-glove with nitrile gloves when handling lysis buffers. Wear ANSI-approved safety glasses and a flame-resistant laboratory coat.
- **Contamination Control:** Wipe pipettes and benches with RNase-away prior to starting. Use only sterile, certified RNase-free pipette tips and microcentrifuge tubes.

3 Materials & Equipment

Table 1: Reagents and laboratory equipment required for spin-column extraction.

Material / Equipment	Vendor & Catalog Number	Primary Function
Lysis Buffer RLT	Qiagen (Cat #79216)	Cell lysis and RNA stabilization
Silica Membrane Spin Columns	Qiagen RNeasy Mini (Cat #74104)	High-efficiency RNA binding
RNase-free DNase I Set	Qiagen (Cat #79254)	In-column digestion of genomic DNA
Ethanol (96–100%)	Sigma-Aldrich (Cat #E7023)	Adjusts binding conditions (70% v/v)
β-Mercaptoethanol	Sigma-Aldrich (Cat #M3148)	Denatures RNases in lysis buffer
Refrigerated Microcentrifuge	Eppendorf 5424 R	Pelleting and spin-wash steps
Nuclease-free Water	Ambion (Cat #AM9937)	Elution of purified total RNA

4 Reagent Preparation

Before beginning the extraction procedure, prepare the following reagents:

- **Lysis Buffer RLT with β-ME:** Add 10 μL of β-ME per 1 mL of Buffer RLT. This solution is stable for up to 1 month at room temperature ($15-25^\circ$C). Prepare under a fume hood.
- **70% Ethanol solution:** Prepare fresh 70% (v/v) ethanol by mixing 35 mL of molecular biology grade 100% ethanol with 15 mL of sterile nuclease-free water.
- **DNase I Stock Solution:** Dissolve lyophilized DNase I (1500 KUnits) in 550 μL of RNase-free water. Gently mix by inverting. Do not vortex. Store aliquots at -20°C.

5 Step-by-Step Procedure

5.1 Cell Harvest and Lysis

1. **Adherent Cells:** Wash with cold PBS. Add 350 μL Buffer RLT directly to the dish, scrape, and transfer lysate to a 1.5 mL tube.
2. **Suspension Cells:** Centrifuge cells at $300 \times g$ for 5 minutes. Resuspend pellet in 350 μL of Buffer RLT.
3. **Homogenization:** Homogenize the lysate by passing it through a sterile 20-gauge needle attached to a 1 mL syringe 5 times, or by centrifuging through a QIAshredder column ($16,000 \times g$, 2 min).

5.2 RNA Binding and On-Column DNase Digestion

1. Add 350 μL 70% ethanol to the homogenized lysate; mix by pipetting. Transfer 700 μL to an RNeasy spin column. Centrifuge at $\geq 8000 \times g$ for 15s; discard flow-through.
2. Add 350 μL Buffer RW1; centrifuge at $\geq 8000 \times g$ for 15s; discard flow-through.
3. Pipette DNase mix (10 μL DNase I stock + 70 μL Buffer RDD, mixed by inversion) directly onto the membrane. Incubate at room temperature for 15 min.
4. Add 350 μL Buffer RW1; centrifuge at $\geq 8000 \times g$ for 15s; discard flow-through.

5.3 Column Washing and Elution

1. Add 500 μL Buffer RPE; centrifuge at $\geq 8000 \times g$ for 15s (discard flow-through). Add another 500 μL Buffer RPE; centrifuge for 2 min.
2. Centrifuge empty column at $16,000 \times g$ for 1 minute to dry the membrane.
3. Place column in a 1.5 mL tube. Add 30–50 μL RNase-free water. Incubate for 1 minute. Centrifuge at $\geq 8000 \times g$ for 1 minute to elute. Store at -80°C.

6 Quality Control / Acceptance Criteria

To ensure the isolation is successful, the eluted RNA must meet the criteria in Table 2.

Table 2: Required quality metrics for RNA integrity and purity.

Quality Metric	Acceptance Range	Method of Assessment
Concentration	≥ 50 ng/μL	UV-Vis Spectrophotometry (Nanodrop)
A_{260}/A_{280} **Ratio**	$1.9 - 2.1$	UV-Vis Spectrophotometry
A_{260}/A_{230} **Ratio**	≥ 2.0	UV-Vis Spectrophotometry
RNA Integrity Number (RIN)	≥ 8.0 (High Quality)	Bioanalyzer / TapeStation Electrophoresis
Genomic DNA Contamination	Not detectable	Negative control PCR (no RT)

7 Waste Disposal & Cleanup

1. **Guanidinium Waste:** Collect flow-through from steps 5.2 and 5.3 in organic waste. Do not mix with bleach.
2. **Solid Waste:** Dispose of pipette tips and tubes in biohazard solid waste for autoclaving.
3. **Spills:** Clean Buffer RLT spills with 10% laboratory detergent, followed by water. Do not use bleach.

8 Revision History

Table 3: SOP revision history log.

Version	Effective Date	Summary of Changes	Author
1.0	May 15, 2024	Initial release of standard protocol.	Dr. Sarah Jenkins
2.0	Aug 20, 2025	Added on-column DNase I digestion step.	Dr. Sarah Jenkins
2.1	Oct 12, 2026	Updated quality control metrics for RNA-seq compatibility.	Dr. Sarah Jenkins