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RNA Isolation Using QIAzol-RNeasy Mini Kit and cDNA Synthesis

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We use this protocol and it's working

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Abstract

This protocol outlines a streamlined workflow for isolating high-quality RNA using QIAzol and RNeasy Mini columns, followed by cDNA synthesis with the High-Capacity cDNA Reverse Transcription Kit. It covers cell harvesting, QIAzol lysis, phase separation, column purification, and RNA before reverse transcription. The method provides reproducible RNA recovery and reliable cDNA generation for downstream gene-expression applications.

Materials

Materials & Reagents

- QIAzol Lysis Reagent
- RNeasy Mini Kit (spin columns, buffers RWT, RPE, collection tubes)
- Chloroform
- 100% ethanol
- PBS (RNase-free)
- Trypsin (0.1–0.25%)
- High-Capacity cDNA Reverse Transcription Kit (ThermoFisher)
- RNase-free water
- RNase-free tubes and pipette tips

Equipment

- Centrifuge capable of 12,000 × g
- Microcentrifuge
- 37°C block or thermal cycler
- Vortex mixer
- RNase-free hood recommended

PART I: RNA ISOLATION (QIAzol-RNeasy Mini Kit)

1 **Suspension Cells (≤1 × 10^7 cells)**

- Count the cells.
- Pellet cells at **300 × g for 5 min**.
- Carefully aspirate **all** supernatant

Note

Remaining medium inhibits lysis and reduces RNA yield.

2 **Adherent Cells (≤1 × 10^7 cells)**

2.1 **Trypsinize and pellet**

- Count cells.
- Aspirate medium, wash once with PBS.
- Add **0.1–0.25% trypsin** until cells detach.
- Add medium (with serum) to neutralize trypsin.
- Transfer to RNase-free tube and spin **300 × g for 5 min**.
- Aspirate supernatant completely.

Note

Medium carryover dilutes QIAzol and lowers RNA yield.

3 **For pelleted cells:**

- Loosen pellet by flicking.
- Add **700 µL QIAzol Lysis Reagent**.
- Vortex or pipette to mix thoroughly.

For direct lysis in dish:

- Add **700 µL QIAzol** directly to dish.
- Scrape using a cell scraper.

- Transfer lysate to RNase-free tube and vortex to remove clumps.

4 Add 140 µL chloroform.

5 Cap and shake vigorously 15 sec.

6 Incubate 2–3 min at RT.

7 Centrifuge 15 min at 12,000 × g, 4°C.

8 Phases after spin: Upper aqueous phase (~350 µL): RNA

9 Transfer the aqueous phase to a new tube.

10 Add 1.5 volumes 100% ethanol (≈525 µL).

11 Mix well by pipetting (precipitate may form).

12 Load ≤700 µL into RNeasy spin column.

13 Spin 15 sec at 8000 × g.

14 Discard flow-through and reuse collection tube.

15 Repeat until entire sample is loaded.

16 Add 700 µL Buffer RWT, spin 15 sec.

17 Add 500 µL Buffer RPE, spin 15 sec.

18 Add 500 µL Buffer RPE, spin 2 min to dry membrane.

Note

Important: Avoid column contact with flow-through to prevent ethanol carryover.

19 Transfer column to new tube and spin 1 min full speed to remove residual buffer.

20 Transfer column to 1.5 mL RNase-free tube.

21 Add 30–50 µL RNase-free water directly onto membrane.

22 Spin 1 min at 8000 × g.

23 For high yield (>30 µg), repeat elution.

24 For higher concentration, elute second time using first eluate (15–30% lower final yield).

PART II: cDNA SYNTHESIS (High-Capacity cDNA Kit)

25 **Prepare 2X RT Master Mix (per 20 µL reaction)**

	Component	With RNase Inhibitor	Without RNase Inhibitor
	10X RT Buffer	2.0 µL	2.0 µL
	25X dNTP Mix (100 mM)	0.8 µL	0.8 µL
	10X RT Random Primers	2.0 µL	2.0 µL

	Component	With RNase Inhibitor	Without RNase Inhibitor
	MultiScribe Reverse Transcriptase	2.0 µL	2.0 µL
	RNase Inhibitor	1.0 µL	-
	Nuclease-free water	3.2 µL	4.2 µL
	Total	10 µL	10 µL

Prepare on ice.

26 Pipette 10 µL of 2X RT master mix into each tube.

27 **Dilute the RNA to the desired input amount (e.g., 0.1–1.0 µg total RNA) in a final volume of 10 µL using RNase-free water.**
Mix gently and keep on ice until adding to the 2X RT master mix.

28 Quick spin to collect contents and remove air bubbles.

29 Keep on ice until loading thermal cycler.

30 Set reaction volume: **20 µL**

	Step	Temperature	Time
	1	25°C	10 min
	2	37°C	120 min
	3	85°C	5 min
	4	4°C	Hold

Start the run.