Jun 25, 2025

Total RNA Extraction from Adherent Mammalian Cancer Cells using the Zymo Quick-RNA Miniprep Kit

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvwqxq7vmk/v1

Boluwatife Afolabi[1]

[1]University of Minnesota

Boluwatife OLU OLU Afolabi
University of Minnesota

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.e6nvwqxq7vmk/v1

Protocol Citation: Boluwatife Afolabi 2025. Total RNA Extraction from Adherent Mammalian Cancer Cells using the Zymo Quick-RNA Miniprep Kit. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwqxq7vmk/v1

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working very well.

Created: June 25, 2025

Last Modified: June 25, 2025

Protocol Integer ID: 220986

Keywords: RNA Extraction, Epithelial Cells, Cancer Cell Line, RT-qPCR, total rna extraction from adherent mammalian cancer cell, total rna extraction, rna miniprep kit this protocol detail, rna miniprep kit, quality total rna from cultured adherent, rna suitable for sensitive downstream application, total rna, purified rna, adherent mammalian cancer cell, rna, epithelial cancer cell, zymo quick, column dnase, extraction, direct cell lysis in the culture plate, direct cell lysi

Funders Acknowledgements:
NIH/NIDCR
Grant ID: R21DE029337

Abstract

This protocol details a streamlined method for extracting high-quality total RNA from cultured adherent epithelial cancer cells using the Zymo Quick-RNA Miniprep Kit. The procedure incorporates direct cell lysis in the culture plate and an efficient in-column DNase I digestion step, yielding purified RNA suitable for sensitive downstream applications such as RT-qPCR and RNA sequencing.

Materials

Apparatus and Materials

Microcentrifuge (capable of ≥ 13,000 x g)
Vacuum aspiration system
Micropipettes (P1000, P200, P20)
Vortex mixer (optional)
Incubator or heat block set to room temperature (20-25°C)Consumables:
Zymo Quick-RNA Miniprep Kit, including:
Zymo-Spin IICR Columns (Green)
Zymo-Spin IIICG Columns (Yellow)
Collection Tubes (1.5 mL or 2 mL)RNase/DNase-free 1.5 mL microcentrifuge tubes for final elution
RNase/DNase-free filtered micropipette tips (1000 µL, 200 µL, 20 µL)
Adherent cells cultured in 6-well or 12-well plates

Reagents

From Zymo Kit:
RNA Lysis Buffer
RNA Prep Buffer
RNA Wash Buffer
DNase I (250 U)
DNA Digestion Buffer

User-Supplied:
Ethanol (EtOH), 200 proof (100%) or ≥95%
DNase/RNase-Free Water

Safety warnings

To prevent irreversible sample degradation, maintain strict RNase-free technique throughout the entire procedure and refrain from talking, as ubiquitous RNases present in aerosols can easily contaminate and destroy your RNA.

Cell Lysis

1 Aspirate the culture medium from the well of the multi-well plate using a vacuum suction system, leaving the adherent cell monolayer intact.

2 Immediately add 500 µL of RNA Lysis Buffer directly to the cell monolayer in each well.

3 Incubate at room temperature for 2 to 10 minutes to ensure complete cell lysis.

4 Mechanically disrupt the cells by scraping the surface of the well with a 1000 µL micropipette tip. Pipette the lysate up and down several times to homogenize.

5 Transfer the entire volume of the lysate from each well into a distinctly labeled RNase-free microcentrifuge tube.

Initial RNA Binding and Filtration

6 Transfer the homogenized lysate into a Zymo-Spin IIICG Column (yellow) pre-seated in a collection tube.

7 Centrifuge at 13,000 x g for 30 seconds.

RNA Precipitation and Binding

8 To the retained flow-through, add an equal volume (1:1 ratio) of 100% ethanol. For example, if you have 500 µL of flow-through, add 500 µL of ethanol.

9 Mix thoroughly by pipetting or vortexing.

10 Transfer the entire mixture into a Zymo-Spin IICR Column (green) pre-seated in a collection tube.

11 Centrifuge at 13,000 x g for 30 seconds.

In-Column DNase I Digestion

12 Add 400 µL of RNA Wash Buffer to the green column.

13 Centrifuge at 13,000 x g for 30 seconds and discard the flow-through.

14 Prepare the DNase I digestion master mix: In a separate, clean tube, combine 75 µL of DNA Digestion Buffer and 5 µL of DNase I enzyme per sample. Mix gently by flicking the tube.

15 Carefully apply the entire 80 µL of the DNase I master mix directly onto the surface of the column matrix.

16 Incubate the column at room temperature for 15 minutes to allow for DNA digestion.

Washing and Purification

17 Following the incubation, add 400 µL of RNA Prep Buffer to the column.

18 Centrifuge at 13,000 x g for 30 seconds. Discard the flow-through.

19 Add 700 µL of RNA Wash Buffer to the column.

20 Centrifuge at 13,000 x g for 30 seconds. Discard the flow-through.

21 Add another 400 µL of RNA Wash Buffer to the column.

22 Centrifuge at 13,000 x g for **1 minute** to facilitate the removal of residual wash buffer.

23 To ensure the column is completely dry, transfer the green column to a new collection tube and centrifuge at top speed (≥13,000 x g) for an additional 15 seconds.

RNA Elution

24 Carefully transfer the Zymo-Spin IICR Column (green) into a new, labeled 1.5 mL RNase/DNase-free microcentrifuge tube.

25 Add 100 µL of DNase/RNase-Free Water directly to the column matrix.

26 Incubate for 1 minute at room temperature.

27 Centrifuge at 13,000 x g for 30 seconds to elute the purified RNA.

28 The eluted RNA is now in the microcentrifuge tube and is ready for quantification and downstream applications.

Protocol references

Quick-RNA Miniprep Kit. *ZYMO RESEARCH* https://www.zymoresearch.com/collections/quick-rna-kits/products/quick-rna-miniprep-kit (2025).