Feb 16, 2025

RNA extraction from Cells

DOI
https://dx.doi.org/10.17504/protocols.io.6qpvr9r2zvmk/v1

Yujie Fan[1]

[1]Caltech

Chelsie Steele
Caltech

DOI: https://dx.doi.org/10.17504/protocols.io.6qpvr9r2zvmk/v1

Protocol Citation: Yujie Fan 2025. RNA extraction from Cells . **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvr9r2zvmk/v1

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

Created: January 08, 2025

Last Modified: February 16, 2025

Protocol Integer ID: 117858

Keywords: rna extraction from cell, rna extraction, rna, extraction, cell

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-020495

Abstract

RNA extraction from Cells

Materials

Direct-zol RNA Miniprep kit (Cat No. R2051)
Trizol (Cat No.R2051, Zymo Research)
DPBS

Collecting Cells

1 Remove cells from the incubator. This protocol is for a 24-well plate.

2 Aspirate the cell media and wash the cells gently once with DPBS.

3 Aspirate the DPBS and add 350ul Trizol (Cat No.R2051, Zymo Research) onto the cells directly. Increase the volume of trizol if you have a well plate larger than a 24 well plate.

4 Use P1000 to pipette up and down to collect all the cell lysis. Make sure to go through all the corners The bottom should become clear if all cells have been detached. At this point, the cell lysis can be frozen down in 1.5mL Eppendorf tubes at -80°C, and stored up to 3 days.

RNA extraction

5 Once the cell lysis has been collected in 1.5mL Eppendorf tubes, the rest of the RNA extraction is preformed using the Direct-zol RNA Miniprep kit (Cat No. R2051) and protocol provided by Zymo Research.

6 Use a P1000 pipette to add an equal volume (350uL) of 100% ethanol to each sample. Pipette up and down multiple times to thoroughly mix the ethanol and trizol, and transfer the entire mixture into a Zymo-Spin IICR column. Centrifuge the columns at x10,000g for 1 minute at room temperature. In the meantime, take out DNAse 1 to thaw.

7 Transfer the columns into a new collection tube, and discard the flow-through. Add 400uL of RNA Wash Buffer (with ethanol already added) to each column, and centrifuge at x10,000g for 1 minute at room temperature.

8 In a separate 1.5mL Eppendorf tube, mix 5uL of DNase 1 with 75uL of DNA digestion buffer (per sample). Mix by gentle inversion/pipetting, and add 80uL of this mixture to each sample column. Incubate for 15-20 minutes at room temperature.

9 After incubation, add 400uL RNA Pre-Wash Buffer to each sample, and centrifuge at same conditions. Discard the flow through.

10 Add 700uL of RNA Wash buffer to each column and centrifuge again at same conditions. Discard the flow-through and centrifuge again to ensure complete removal of ethanol and washing buffer.

11 Transfer the columns to pre-labeled RNAse-free Eppendorf tubes. Add 50uL of DNAse/RNAse-free water to each column, and let sit for one minute.

12 Centrifuge at same conditions for one minute. Discard flow-through and nanodrop the RNA concentrations. At this point, RNA can be stored long-term in -80°C.