Mar 09, 2021

Total Cellular RNA Purification Protocol from Animal Tissue (Trizol + RNeasy)

Loyal Goff[1]
[1]Johns Hopkins

1 *Works for me* This protocol is published without a DOI.

gofflab

Loyal Goff
Johns Hopkins

SUBMIT TO PLOS ONE

ABSTRACT

This protocol provides an optimaized, high-quality total RNA extraction method that balances time and RNA-yield effectively by combining the phase separation properties of Trizol with the efficiency of glass fiber filter column binding. Tissue homogenization by mortar & pestle in LN2 ensure minimal RNA degradation and efficient lysis in Trizol.

PROTOCOL CITATION

Loyal Goff 2021. Total Cellular RNA Purification Protocol from Animal Tissue (Trizol + RNeasy). **protocols.io** https://protocols.io/view/total-cellular-rna-purification-protocol-from-anim-bs3mngk6

CREATED

Mar 06, 2021

LAST MODIFIED

Mar 09, 2021

PROTOCOL INTEGER ID

47949

MATERIALS TEXT

- Frozen tissue (50-100mg)
- LN_2 and appropriate container(s)
- Mortar and Pestle (pre-chilled in -80)
- Trizol
- Chloroform
- 70% ethanol
- RNeasy mini-spin columns and kit reagents
- 1.5mL eppendorf tubes (RNase/DNase Free)
-

Sample Homogenization

1 Begin with **50-100 mg** tissue stored at **-80 °C** (for each **1 mL** TRIzol you will use).

Place tissue in liquid nitrogen and grind to a powder with a frozen mortar and pestle.

2

3 Decant powder and liquid nitrogen into an RNAse-free frozen microcentrifuge tube (using a frozen spatula as a guide).

4 Liquid nitrogen will evaporate, but prior to tissue thawing, add **1 mL** TRIzol andvortex to mix well.

5 Pulse spin to collect contents, and place on ice.

Phase Separation 20m 5s

6 Add **100 µl** of chloroform to each tube and vortex each sample for **00:00:05** (or until cloudy). Place sample on ice for **00:05:00** . Centrifuge at **13.000 rpm** for **00:15:00** at **4 °C** . 20m 5s

7 Remove about **250 µl** of the upper aqueous phase from each tube and place it in a new tube. While removing sample, be sure to completely avoid the interface and the organic phase. Save the organic phase if isolation of DNA or protein is desired (as per TRIzol package instructions).

8 Slowly add **250 µl** (in **50 µl** aliquots) of 70 % ethanol at **25 °C** to tube (equal parts 70% ethanol and aqueous phase). Mix thoroughly after each aliquot.

RNA consolidation 15s

9 Apply sample to RNeasy mini-spin column in 2mL collection tube.

10 Centrifuge at r **25 °C** for **00:00:15** at **10.000 rpm** , and discard flow- through. 15s

Wash 2m 30s

11 Pipette **700 µl** of RW1 onto the column.

12 Centrifuge at **25 °C** for **00:00:15** at **10.000 rpm** . 15s

13 Discard flow-through and collection tube.

14 Put column in new 2mL collection tube.

15 Pipette **500 µl** RPE solution (**100 µl** RPE in **400 µl** 100% ethanol) on column. Centrifuge for **00:00:15** at **10.000 rpm** and discard flow-through. 15s

16 Pipette **500 µl** RPE solution (**100 µl** RPE in **400 µl** 100% ethanol) on column Centrifuge for **00:02:00** at maximum speed. 2m

Elution 11m

17 Transfer RNeasy column into new 1.5mL collection tube.

18 Slowly pipette **50 µl** pre-warmed (**50-65 °C**) RNase-free water onto the center of the RNeasy membrane.

18.1 Wait **00:10:00** (incubation needed only if yields are low). 10m

19 Centrifuge for **00:01:00** at **10.000 rpm** 1m

20 Repeat steps 18 and 19 as needed to fully elute RNA from column.

21 Once off column, place eluate (RNA) on ice. Store at **-80 °C** .