Nuclear RNA purification V.2

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Version 2
Sep 21, 2021
This protocol is published without a DOI.

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ABSTRACT

Protocol for purifying nuclear RNA for qRT-PCR or next generation sequencing analysis.

PROTOCOL CITATION

Michael Tellier 2021. Nuclear RNA purification. **protocols.io** https://protocols.io/view/nuclear-rna-purification-byfhptj6 Version created by Michael Tellier

KEYWORDS

RNA, purification, cellular fractionation, sub-cellular, nucleus

CREATED

Sep 21, 2021

LAST MODIFIED

Sep 21, 2021

PROTOCOL INTEGER ID

53449

Day 1

1 1. Split the cells to obtain a ~80% confluent 10 cm dish on Day 2.

Day 2

2 **RNA extraction**

1. Wash the cells twice with ice-cold PBS.
2. Scrap the cells in 1.2 ml of ice-cold PBS.
3. Centrifuge at 1,000 rpm for 5 minutes at 4°C.
4. Resuspend the pellet with slow pipetting in 1 ml of Lysis Buffer B (10 mM Tris-HCl pH 8, 140 mM NaCl, 1.5 mM MgCl2, 0.5 % NP-40).
5. Centrifuge at 1,000 g for 3 minutes at 4°C.
6. Resuspend the pellet with slow pipetting in 1 ml of Lysis Buffer B and transfer to an ice-cold 14 ml round-bottom tube.
7. Add drop by drop 100 µl of the Detergent Stock Solution (3.3 % (w/v) sodium deoxycholate, 6.6 % (v/v) Tween 40) under slow vortexing.
8. Transfer to a fresh ice-cold 1.5 ml tube. Centrifuge at 1,000 g for 3 minutes at 4°C.
9. Resuspend the pellet with slow pipetting in 1 ml of Lysis Buffer B.

10. Centrifuge at 1,000 g for 3 minutes at 4°C.
11. Resuspend the pellet in 1 ml of TRIzol using a 21-gauge syringe and incubated 5 minutes at room temperature.
12. Add 200 µl of chloroform and vortex the sample vigorously for 15 seconds.
13. Centrifuge at 12,000 g for 15 minutes at 4°C.
14. Transfer the aqueous fraction to a new tube containing 580 µl of isopropanol.
15. Incubate 10 minutes at room temperature.
16. Centrifuge at 12,000 g for 10 minutes at 4°C.
17. Remove most of the supernatant with a 1 ml pipette.
18. Centrifuge at 12,000 g for one minute at 4°C.
19. Remove the remaining liquid with a 10 or 20 µl tip.
20. Resuspend the pellet in 87 µl of RNase-free water, 10 µl of 10 × DNase buffer, 2 µl of DNase I, and 1 µl of RNase OUT.
21. Incubate for 30 minutes at 32°C.
22. Add 100 µl of acid-phenol:chloroform pH 4.2.
23. Vortex 10 seconds, then centrifuge 5 minutes at 13,000 rpm at room temperature.
24. Transfer the upper phase to a new tube and add 100 µl of acid-phenol:chloroform pH 4.2.
25. Vortex 10 seconds, then centrifuge 5 minutes at 13,000 rpm at room temperature.
26. Transfer the upper phase to a new tube and add 250 µl of 100% ethanol, 10 µl of NaOAc, and 1 µl of Glycoblue (or 1 µl of glycogen 10 mg/ml).
27. Invert the tube several times and incubate at -20°C for at least two hours (or overnight).
28. Centrifuge for 20 minutes at 13,000 rpm at 4°C.
29. Remove the supernatant and centrifuge for two minutes at 13,000 rpm at 4°C.
30. Remove the last drops, air dry for 1-2 minutes, and resuspend in 20 ul of RNase-free water.
31. Determine the concentration and the 260/280 and 260/230 ratios using a NanoDrop or another system.
32. Perform the cDNA reaction for qRT-PCR or NGS.