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RNA cellulose-based purification

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We use this protocol and it's working

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Abstract

Protocol for RNA purification

Materials

Materials needed:
Corning 50 mL conical from newly opened bag
RNA-only filter pipet tips
Individually-wrapped serological pipets
RNase Zap (or equivalent)
Spatula/spoonlet sprayed with RNase Zap
Weigh boats sprayed with RNase Zap
HEPES 1M buffer solution, molecular-biology grade (83264, Sigma-Aldrich)
EDTA 0.5M solution, molecular-biology grade (324506, Millipore Sigma)
NaCl 5M solution, molecular-biology grade (S5150, Sigma-Aldrich)
Ethanol, 200 proof, molecular-biology grade
Water, molecular-biology grade
Cellulose fibers (C6288, Sigma-Aldrich)
NucleoSpin Filters (740606, Macherey-Nagel)
Container that holds microcentrifuge tubes, sprayed with RNase Zap

Equipment Needed:
Shaker
Benchtop centrifuge for microcentrifuge tubes
Pipetmans (p2, p20, p200, p1000)
Pipet-Aid
Precision balance scale
Biosafety Cabinet (TC hood)

Cellulose-based Purification of IVT mRNA

1h 33m

1

Note

All procedures should be performed in RNA bench or TC hood wiped down with RNase ZaP, when possible. User should be wearing a lab coat, mask, and RNase-free gloves. The outside surfaces of all equipment (e.g. scale platform & inside glass walls, pipetman, pipet aid, etc.) and tubes should be wiped down with RNase Zap before beginning work.

To make 50mL **chromatography buffer (in TC hood)**:

1. Add **40.24 mL nuclease-free water** to a Corning 50 mL centrifuge tube (preferably from a newly opened package)
2. Add **0.5 mL HEPES** solution (10 mM final conc.) to same tube
3. Add **10 µL EDTA** solution (0.1 mM final conc.) to same tube
4. Add **1.25 mL NaCl** solution (125 mM final conc.) to same tube
5. Add **8 mL ethanol**, 200 proof (16% v/v) to same tube
6. Invert tube 6-8 times to mix.

2 1h 33m

Procedure:

1. Calculate how much cellulose to weigh out
    a. # of RNA samples to be purified x 2 × 0.2 = **grams** of cellulose needed
2. Weigh out calculated cellulose on analytical balance under RNA conditions and add to a 50 mL conical tube
3. Add calculated volume of chromatography buffer (from above) to cellulose tube
    a. (# of RNA samples to be purified x 2) – volume of cellulose (rough estimate) = **mL** of chromatography buffer to add
4. Attach cellulose tube (lying on side – make sure cap is tight!) to vortex platform on RNA bench using a rubber band, switch mode to continuous shaking, and turn it up to notch 2 or 3 so that the tube is vigorously shaking. **Shake for 10 min.**
5. Pipet 700 µL of cellulose slurry to the top of each NucleoSpin column tube
6. Spin NucleoSpin column tubes down at 14,000 x g for 00:01:00 at RT.
7. Discard flowthrough and add 500 µL chromatography buffer to each of the same NucleoSpin column tubes.
8. Place NucleoSpin column tubes in tube holder and attach to the vortex platform using a rubber band. **Shake vigorously for 5 min.** Put half of the tubes to the side (don't spin down).
9. Spin NucleoSpin column tubes down at 14,000 x g for 00:01:00 at RT.

10. Discard flowthrough and add each IVT mRNA diluted in 500 µL chromatography buffer to respective NucleoSpin column tubes containing cellulose.
11. Place NucleoSpin column tubes in tube holder and attach to the vortex platform using a rubber band. **Shake vigorously for 30 min.**
12. Place step 11 NucleoSpin columns into a new collection tube or RNase-free 1.5 mL tube.
13. Spin step 11 NucleoSpin column tubes plus tubes you reserved in step 8 at 14,000 x g for 60 sec at RT.
14. Transfer flow through of step 11 tubes to top of reserved tubes in step 8. After transfer, put step 11 NucleoSpin column tubes on ice in case you want to extract the dsRNA fraction.
15. Place step 14 tubes in tube holder and attach to vortex platform using a rubber band. **Shake vigorously for 30 min.**
16. Place step 14 NucleoSpin columns into a RNase-free 1.5 mL tube
17. Spin step 14 tubes down at 14,000 x g for 00:01:00
18. Add 0.1 vol 3M NaOAc (pH 5.5 ; RNase-free) and 1 vol ice-cold isopropanol to flow-through of step 17
19. Vortex to mix and incubate on ice for 01:00:00
20. Spin down at max speed for 00:20:00 at 4 °C
21. Pipet out supernatant and add 0.5 mL ice-cold 70-75% Ethanol to each tube to wash
22. Spin down at max speed for 00:05:00 at 4 °C
23. Pipet out ethanol and add another 0.5 mL ice-cold 70-75% ethanol to each to for 2nd wash
24. Spin down at max speed for 00:05:00 at 4 °C
25. Pipet out ethanol and dry RNA pellets
26. Add 20-40 µL RNase-free water to each pellet
27. Elute RNA on ice for 2+ hours or overnight in refrigerator
28. Resuspend RNA pellets and spec before using or storing at -80 °C

Protocol references

https://www.cell.com/molecular-therapy-family/nucleic-acids/pdfExtended/S2162-2531(19)30049-6