Isolation of exosomal RNA from cell culture supernatant using the System Biosciences Exoquick Seramir tissue culture kit

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Method Article

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Abstract

This protocol describes how to isolate exosomal RNA from cell culture supernatant using the Exoquick Seramir tissue culture kit.

Introduction

Extracellular RNAs \(exRNAs) have been found to play an important role in intercellular communication in the body. exRNAs are present in biofluids, in complexes with lipoproteins and ribonucleoproteins, and in extracellular vesicles such as exosomes and microvesicles. Further study and characterization of exRNAs and their carriers could lead to identification of new biomarkers and have potential for development of novel therapeutics.

Reagents

Exoquick Seramir tissue culture kit \(Systems Biosciences, RA800-TC1) 100% ethanol \(VWR, 89125-186)

Equipment

Microfuge Microfuge tubes, 1.5 mL

Procedure

1. Harvest cell culture medium from 80% confluent cells grown in serum-free medium. 2. Optional: Spin media at 500-1,000 x g for 10 min. 3. Pre-clear medium by centrifuging at 2000 x g for 10 min, then filter using a 0.22 µm PES Steriflip membrane and freeze at -80°C until needed. 4. Thaw 4 mL of cell culture supernatant on ice. 5. Add 0.8 mL of Exoquick-TC and mix well by inversion 3 times. 6. Incubate at 4°C overnight. 7. Centrifuge at 16,000 x g for 2 min, remove supernatant and keep the exosome pellet. 8. Add 350 µL Lysis buffer to pellet, vortex for 15 s, and incubate at room temperature for 5 min. 9. Add 200 µL of 100% ethanol and vortex for 10 s. 10. Transfer all \(600 µL) to spin column and centrifuge at 16,000 x g for 1 min \(or until the solution has flowed through). 11. Discard flow through. 12. Add 400 µL of wash buffer and centrifuge at 16,000 x g for 1 min, then discard flow-through. 13. Repeat step 12 once more. 14. Discard flow-through and centrifuge at 16,000 x g for 2 min to dry column. 15. Transfer spin column to new collection tube and add 14 µL or 30 µL RNase-free water or Elution buffer directly to the membrane.[1] 16. Centrifuge at 400 x g for 2 min.[2] 17. Centrifuge at 16,000 x g for 1 min.

Troubleshooting

[1] This volume was selected to match that of other kits to enable fair comparisons. As little as 10 µL RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 µL RNase-free water, as the spin column membrane will not be sufficiently hydrated. The dead volume of the RNeasy MinElute spin column is 2

µL: elution with 14 µL RNase-free water results in a 12 µL eluate. [2] Centrifuging at a low speed first helps the solvent wet the surface of the membrane prior to the full speed centrifuging step. This results in a better yield / RNA recovery from the membrane.

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