Isolation of exosomal RNA from urine 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 urine by 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-TC Exosome Precipitation solution \(System Biosciences, EXOTC50A-1) Seramir Exosome RNA Amplification kit \(System Biosciences, RA808A-1) 100% ethanol \(Koptec, V1016) RNase-free water \(Ambion, AM9937)

Equipment

Microfuge Microfuge tubes, 1.5 mL

Procedure

Exosome purification 1. Thaw samples on ice. 2. Add 100 µL of Exoquick-TC precipitation solution to 500 µL of urine in a 1.5 mL microfuge tube. 3. Mix well by inversion 3 times. 4. Incubate overnight at 4°C. 5. Centrifuge ExoQuick/urine mixture at 16,000 x g for 2 min. 6. Remove supernatant by pipetting \(leaving behind 50 µL). Keep the exosome pellet. 7. Add 350 µL Lysis buffer to exosome pellet and vortex 15s. 8. Place at room temperature for 5 min \(to allow complete lysis). **exoRNA purification** 9. Add 200 µL of 100% ethanol. 10. Vortex for 10s. 11. Assemble spin column and collection tube. 12. Transfer all \(600 µL) to spin column. 13. Centrifuge at 16,000 x g for 1 min. \(Spin longer if the solution has not flowed through.) 14. Discard flow-through. 15. Add 400 µL wash buffer. 16. Centrifuge at 16,000 x g for 1 min. 17. Repeat steps 15 and 16 once more. **exoRNA elution** 18. Discard flow-through. 19. Centrifuge at 16,000 x g for 2 min to dry. 20. Transfer spin column to fresh collection tube. 21. Add 14 µL RNase-free water directly to membrane in spin column.[1] 22. Centrifuge at 400 x g for 2 min.[2] 23. Increase speed to 16,000 x g and centrifuge for 1 min.

Troubleshooting

[1] This volume was selected to match that of other kits to enable fair comparisons. [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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