Isolation of exosomal RNA from urine using ultracentrifugation and the Qiagen miRNeasy Micro kit

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Method Article

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Abstract

This protocol describes how to isolate exosomal RNA from urine by using ultracentrifugation for exosomal enrichment followed by the Qiagen miRNeasy Micro kit for RNA isolation.

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

Urine \(pre-cleared by centrifugation at 2000 x g for 10 min) Phosphate-buffered saline \(PBS)

Equipment

Ultracentrifuge \(Beckman Coulter, Optima MAX – XP Ultracentrifuge) Swinging bucket rotor \(MLS-50) Ultracentrifuge tubes \(Beckman Coulter, 326819)

Procedure

1. Thaw samples on ice. 2. Transfer 0.5 mL of urine into an ultracentrifuge tube. 3. Bring up volume to fill the ultracentrifuge tube with PBS \(3 mL). 4. Centrifuge at 100,000 x g for 70 min. 5. Discard 3 mL of supernatant. 6. Add 3 mL of fresh PBS. 7. Centrifuge at 100,000 x g for 70 min. 8. Remove and discard 3.3 mL of supernatant. 9. Resuspend the leftover solution \(usually about 200 µL). 10. Freeze at -80°C until needed. 11. Isolate RNA using the miRNeasy Micro kit.

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