Extracellular RNA isolation using the SeraMir Kit

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Method Article

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Abstract

This protocol describes how to isolate extracellular RNA from exosomes using the SeraMir Kit in order to detect, identify and quantify the extracellular RNA.

Introduction

Extracellular RNAs \(exRNAs) have been identified in every biofluid that has been tested. They have been found in extracellular vesicles, ribonucleoprotein complexes and lipoprotein complexes. exRNAs are interesting because they may serve as signalling molecules between cells, they have the potential to serve as biomarkers for prediction and diagnosis of disease, and exRNAs or the extracellular particles that carry them might be used for therapeutic purposes. The Sample and Assay Standards Working Group of the Extracellular RNA Communication Consortium \(ERCC) is a group of laboratories funded by the U.S. National Institutes of Health to develop robust and standardized methods for collecting and processing of biofluids, separating different types of exRNA-containing particles and isolating and analyzing exRNAs. In our first joint endeavour, we held a series of conference calls and in-person meetings to survey the methods used among our members, placed them in the context of the current literature and used our findings to identify areas in which the identification of robust methodologies would promote rapid advancements in the exRNA field. A full list of the protocols developed during this effort is available at the exRNA Portal, the ERCC's website \("http://exrna.org/resources/protocols/":http://exrna.org/resources/protocols/). This protocol for isolating extracellular RNA from exosomes using the SeraMir Kit is one of the RNA isolation methods compared in "the associated publication":http://www.journalofextracellularvesicles.net/index.php/jev/article/view/26533.

Reagents

SeraMir Exosome RNA Purification Column kit \(System Biosciences, catalog # RA808A-1) ethanol

Equipment

Microfuge Microfuge tubes, 1.5 mL

Procedure

1. Add 350 µl LYSIS Buffer to resuspended exosome pellet and vortex 15 seconds. 2. Incubate for 5 minutes at room temperature for 5 minutes. 3. Add 200 µl of 100% Ethanol. 4. Vortex 10 seconds. 5. Assemble spin column and collection tube. Transfer mixture \(600 µl) to spin column. 6. Centrifuge for 1 minute at 13,000 rpm at room temperature. \(Check to see that the liquid has all flowed through; if not, spin longer.) 7. Discard flow-through. 8. Add 400 µl WASH Buffer. 9. Centrifuge for 1 minute at 13,000 rpm at room temperature. \(Check to see that the liquid has all flowed through; if not, spin longer.) 10. Discard

flow-through. 11. Add 400 µl WASH Buffer. 12. Centrifuge for 1 minute at 13,000 rpm at room temperature. \(Check to see that the liquid has all flowed through; if not, spin longer.) 13. Discard flow-through. 14. Centrifuge for 2 minutes at 13,000 rpm at room temperature. 15. Transfer spin column to fresh microfuge tube. 16. Add 30 µl ELUTION Buffer directly to membrane in spin column. 17. Centrifuge for 2 minutes at 2,000 rpm at room temperature \(loads buffer into membrane). 18. Centrifuge for 1 minute at 13,000 rpm at room temperature to elute RNA.

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This protocol was modified from the manufacturer’s instructions for the SeraMir Exosome RNA Purification Column kit.