Exosome isolation from plasma using ExoQuick reagent

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Method Article

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Abstract

This protocol describes how to isolate exosomes from plasma using the ExoQuick reagent in order to detect, identify and quantify 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 isolation of exosomes from plasma using the ExoQuick reagent is one of the methods for extracellular vesicle \(EV) and particle enrichment compared in "the associated publication":http://www.journalofextracellularvesicles.net/index.php/jev/article/view/26533.

Reagents

phosphate-buffered saline \(PBS)

Equipment

ExoQuick plasma prep and Exosome precipitation kit \(System Biosciences, catalog # EXOQ5TM-1)
Microfuge Microfuge tubes, 1.5 mL

Procedure

1. Transfer 200 µl of plasma into a 1.5 ml microfuge tube. 2. Add 2 µL of Thrombin \(500U/mL) to a final concentration of 5U/mL. 3. Incubate at room temperature for 5 minutes while mixing \(gently flicking tube). 4. Centrifuge for 5 minutes at 10,000 rpm. There should be a visible fibrin pellet at the bottom of the tube. 5. Transfer supernatant to a fresh tube. 6. Add 50 µl of ExoQuick Exosome Precipitation Solution to the plasma and incubate 30 min at 4°C. 7. Centrifuge ExoQuick/plasma mixture at 1,500 × g for 30

minutes at room temperature. 8. Aspirate supernatant. 9. Spin down residual ExoQuick solution by centrifugation at 1,500 × g for 5 minutes at room temperature. 10. Remove all traces of fluid by aspiration, taking great care not to disturb the pellet. 11. Resuspend the pellet in 20 µl sterile PBS.