Enrichment of extracellular vesicles from serum or plasma via ultracentrifugation

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Method Article

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Abstract

This protocol describes how to use differential ultracentrifugation to enrich extracellular vesicles \(EVs) from serum or plasma in order to detect, identify and quantify extracellular RNA from those EVs.

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 enrichment of extracellular vesicles \(EVs) from serum or plasma via ultracentrifugation is one of the methods for EV and particle enrichment compared in "the associated publication":http://www.journalofextracellularvesicles.net/index.php/jev/article/view/26533.

Procedure

1. For each replicate, start with 1 ml serum or plasma. 2. Bring up volume to fill ultracentrifuge tube with PBS. 3. Centrifuge for 90 min at 100,000 x g at 4°C. 4. Discard supernatant and resuspend pellet in PBS to fill ultracentrifuge tube. 5. Centrifuge for 70 min at 100,000 x g at 4°C. 6. Discard supernatant. 7. Proceed to miRNeasy RNA isolation by adding 1 mL Qiazol to pellet.