

Exosome isolation from serum using ExoQuick reagent

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Method Article

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Abstract

This protocol describes how to isolate exosomes from serum using the ExoQuick Plasma Prep and Exosome Precipitation kit. It supercedes an earlier protocol (<http://dx.doi.org/10.1038/protex.2015.109>). The new protocol is based on our experience vetting the original protocol in multiple labs. The major change is an increase in input volume of biofluid from 200 μ L to 500 μ L. We found that the larger input volume led to more reproducible amounts of RNA isolation across multiple experiments. We have also included footnotes to explain changes we made to manufacturers' protocols, and observations that were made while carrying out the protocols.

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 Plasma Prep and Exosome Precipitation kit (System Biosciences – EXOQ5TM-1) Phosphate-buffered saline (PBS)

Equipment

Microfuge 1.5 mL Microfuge tubes

Procedure

1. Transfer 500 μ L of serum into a 1.5 ml microfuge tube.
2. Add 125 μ L of ExoQuick Exosome Precipitation Solution to the serum and incubate for 30 minutes at 4°C.
3. Centrifuge ExoQuick/serum mixture at $1,500 \times g$ for 30 minutes at room temperature.
4. Aspirate supernatant.
5. Spin down residual ExoQuick solution by centrifugation at $1,500 \times g$ for 5 minutes at room temperature.
6. Remove all traces of fluid by aspiration, taking great care not to disturb the pellet.
7. Resuspend the pellet in 50 μ L sterile PBS and proceed with RNA isolation using SeraMir Exosome RNA Purification Column Kit.

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