Isolation of exosomal RNA from serum or plasma using ultracentrifugation and the Qiagen miRNeasy Micro kit

CURRENT STATUS: POSTED

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DOI:

10.1038/protex.2017.079

SUBJECT AREAS

Cell biology *Biological techniques*

KEYWORDS

exRNA, extracellular vesicles, exosomes, serum, plasma, blood, ultracentrifugation, RNA isolation, miRNeasy kit

Abstract

This protocol describes how to isolate exosomal RNA from serum/plasma by using ultracentrifugation for exosomal enrichment followed by the Qiagen miRNeasy Micro kit for RNA isolation. It supercedes an earlier protocol (http://dx.doi.org/10.1038/protex.2015.111). The new protocol is based on our experience vetting the original protocol in multiple labs. The major change is a decrease in input volume of biofluid from 1 mL to 500 µL in order to match the input volume of other protocols the ERC consortium is developing in tandem.

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

Qiagen miRNeasy micro kit (217084)

Chloroform (Sigma-Aldrich – 319988)

100% ethanol (Koptec – V1016)

70% ethanol

RNase-free water (Ambion – AM9937)

Phosphate-buffered saline (PBS)

Equipment

Ultracentrifuge

Swinging bucket rotor (TLA110/MLA5)

Microfuge

1.5 mL Microfuge tubes

Vortexer

Phase lock gel tubes, 2 mL (VWR – 10847-802)

Procedure

1. Start with 500 µL serum or plasma.

2. Bring up volume to fill ultracentrifuge tube (2.3-2.5 mL) with PBS.
3. Centrifuge for 70 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. Resuspend pellet in 50 µL PBS and store at -80°C, or
8. Proceed to miRNeasy RNA isolation by adding 700 µL Qiazol to pellet.

Acknowledgements

PN, AP and RG were supported by NIH grant UH2-UH3 TR000890. SS and LL were supported by U01 HL126494 and UH3 TR000906. XZ, LB and XOB were supported by U19 CA179463.