Isolation of exosomal RNA from serum or plasma using the New England Peptide ME™ kit

Louise C. Laurent (llaurent@ucsd.edu)
Department of Reproductive Medicine, University of California, San Diego, La Jolla, CA, USA

Roopali Gandhi (rgandhi@rics.bwh.harvard.edu)
Department of Neurology, Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Parham Nejad
Department of Neurology, Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Anu Paul
Department of Neurology, Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Srimeenakshi Srinivasan
Department of Reproductive Medicine, University of California, San Diego, La Jolla, CA, USA

Method Article

Keywords: exRNA, extracellular vesicles, exosomes, serum, plasma, blood, ME kit

Posted Date: June 16th, 2017

DOI: https://doi.org/10.1038/protex.2017.074

Abstract

This protocol describes how to isolate exosomal RNA from serum or plasma by using the New England Peptide ME kit 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.2015.113). 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 µL to 500 µL. 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

New England Peptide ME^{TM} kit – ME-010-kit Phosphate-buffered saline \(PBS), pH 7.4 Protease Inhibitor Cocktail, Set III EDTA-free \(EMD) - 539134 Qiagen miRNeasy micro kit - 217084 Chloroform \(Sigma-Aldrich, 319988) 100% ethanol \(Koptec, V1016) 70% ethanol RNase-free water \(Ambion – AM9937)

Equipment

Microfuge 1.5 mL Microfuge tubes Phase lock gel tubes, 2 mL \(VWR, 10847-802) Vortexer

Procedure

1. Reconstitute the Vn96 peptide to 2.5 µg/µL by adding 200 µL ME-buffer. Reconstitute the negative control Vn96-Scr peptide by adding 40 µL ME-buffer. Store at 4°C. 2. Transfer 500 µL of serum or plasma into a 1.5 mL microfuge tube. 3. Add 500 µL PBS. 4. Mix thoroughly. 5. Add 5 µL protease inhibitor cocktail. 6. Centrifuge for 7 minutes at 10,000 x g at room temperature to remove debris. 7. Transfer supernatant to fresh tube, avoiding any pelleted material. 8. Add 20 µL reconstituted Vn96 peptide \(use same amount of Vn96-Scr as a negative control). 9. Invert tube 10 times. 10. Incubate at room temperature for 30 minutes on a rotator.[1] 11. Centrifuge for 7 minutes at 10,000 x g at room temperature to pellet extracellular vesicles. 12. Carefully remove and discard supernatant. 13. Wash by adding 1 mL PBS + 5 µL protease inhibitor cocktail, inverting 10 times, centrifuging for 7 minutes at 10,000 x g at room

temperature, and carefully removing and discarding supernatant. 14. Repeat Step 13. 15. Resuspend pellet in 700 µL Qiazol and isolate RNA using miRNeasy micro kit.

Troubleshooting

[1] This is different from the manufacturer's protocol which suggests 15 min at RT or overnight at 4°C.

Acknowledgements

This protocol was modified from the New England Peptide ME kit. PN, AP and RG were supported by NIH grant UH2-UH3 TR000890. SS and LL were supported by U01 HL126494 and UH3 TR000906.