Isolation of extracellular vesicles from serum or plasma using the ME Kit

Louise C. Laurent (llaurent@ucsd.edu)
University of California San Diego, Sanford Consortium for Regenerative Medicine, 2880 Torrey Pines Scenic Drive, La Jolla, CA

Roger P. Alexander (rogerpalexander@gmail.com)
Sample and Assay Standards Working Group of the Extracellular RNA Communication Consortium

Method Article

Keywords: exRNA, serum, plasma, blood, ME

Posted Date: December 21st, 2015

DOI: https://doi.org/10.1038/protex.2015.113

Abstract

This protocol describes how to isolate extracellular vesicles from serum or plasma using the ME Kit from New England Peptide in order to detect, identify and quantify extracellular RNA. ---------- COMMENTS Title and abstract modified. - Ashleigh Carver, Editorial Assistant, Nature Protocols, 14/09/2016

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 extracellular vesicles \(EVs) from serum or plasma using the ME kit from New England Peptide 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

ME Kit \(New England Peptide, catalog # ME-010-kit) phosphate-buffered saline \(PBS), pH 7.4 Protease Inhibitor Cocktail, Set III EDTA-free \(EMD, catalog # 539134)

Equipment

Microfuge Microfuge tubes, 1.5 mL

Procedure

1. Reconstitute 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 200 µl of serum or plasma into a 1.5 ml microfuge tube. 3. Add 800 µl PBS. 4. Mix thoroughly. 5. Add 5 µl protease inhibitor cocktail. 6.

Centrifuge for 7 minutes at 17,000 xg 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 15 minutes on a rotator. 11. Centrifuge for 7 minutes at 10,000 xg 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 xg at room temperature, and carefully removing and discarding supernatant. 14. Repeat wash. 15. Resuspend pellet in 100 µl PBS and continue on to miRNeasy protocol for RNA isolation.

Acknowledgements

This protocol was modified from the manufacturer's instructions for the ME Kit.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement0.pdf