Isolation of extracellular vesicles and RNA from serum or plasma using the ExoRNeasy Mini Kit.

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Method Article

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Abstract

This protocol describes how to isolate extracellular vesicles \(EVs) and extracellular RNA \(exRNA) from serum or plasma using the ExoRNeasy Mini Kit in order to detect, identify and quantify exRNA. ---------- 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 EVs and exRNA from serum or plasma using the ExoRNeasy Mini Kit is one of the EV / RNA isolation methods compared in the associated publication. \("http://www.journalofextracellularvesicles.net/index.php/jev/article/view/26533":http://www.journalofextracellularvesicles.net/index.php/jev/article/view/265)

Reagents

ExoRNeasy Serum/Plasma Mini Kit \(Qiagen) chloroform 70% ethanol 100% ethanol RNase-free water

Equipment

Phase Lock Heavy PLG tube \(5Prime, catalog # 2302830) Microfuge Microfuge tubes, 1.5 mL

Procedure

1. Transfer 200 µl of serum or plasma into a 1.5 ml microfuge tube. 2. Add 200 µl of buffer XBP. 3. Invert 5 times. 4. Add the sample/XBP mixture onto the exoEasy spin column. 5. Centrifuge for 1 minute at 500 xg at room temperature. 6. Discard flow-through. 7. Add 10 ml of buffer XWP to the exoEasy spin column. 8. Centrifuge for 5 minutes at 5,000 xg at room temperature. 9. Transfer the spin column to a fresh collection tube. 10. Add 700 µl Qiazol to the membrane of the spin column. 11. Centrifuge for 5 minutes at 5,000 xg at room temperature. 12. Transfer the flow-though, which is the lysate, to a PLG tube.[1] 13. Vortex for 5 seconds.[2] 14. Incubate at room temperature for 5 minutes. 15. Add 90 µl chloroform. 16. Shake vigorously for 15 seconds. 17. Incubate for 3 min at room temperature. 18. Centrifuge sample for 5 min at 12,000 x g at 4°C. 19. Transfer the upper aqueous phase to a new microcentrifuge tube. 20. Carefully measure the aqueous phase and add 2 volumes of 100% ethanol. 21. Mix gently and thoroughly. Do not centrifuge and do not delay moving on to the next step. 22. Assemble a MinElute spin column in a new collection tube. 23. Load up to 700 µl of the mixture from step 5, including any precipitate that may have formed, onto the column. 24. Centrifuge for 15 seconds at 1,000 x g at room temperature.[3] 25. Discard flow-through. 26. Repeat steps 23-25 until entire sample has been loaded. 27. Add 700 µl of buffer RWT to the RNeasy MinElute spin column. 28. Centrifuge for 15 seconds at 8000 x g at room temperature to wash the column. 29. Discard the flow-through. 30. Pipet 500 µl Buffer RPE onto the RNeasy MinElute spin column. 31. Centrifuge for 15 seconds at 8000 x g to wash the column. 32. Discard the flow-through. 33. Pipet 500 µl Buffer RPE onto the RNeasy MinElute spin column. 34. Centrifuge for 2 minutes at ≥ 8000 x g \(≥10,000 rpm) at room temperature to wash the column. 35. Discard the collection tube with the flow-through.[4] 36. Transfer the RNeasy MinElute spin column into a new 2 ml collection tube \(supplied). 37. Open the lid of the spin column, and centrifuge at full speed \(14,000 xg) for 5 min to dry the membrane.[5] 38. Discard the collection tube with the flow-through.[4] 39. Transfer the RNeasy MinElute spin column into a new 1.5 ml collection tube \(supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane.[6] 40. Centrifuge for 1 minute at full speed to elute the RNA.

Timing

less than an hour

Troubleshooting

1) The PLG tube is not essential, but it does make it easier to remove the aqueous phase without contamination with the interphase. 2) A spike-in control can be added here. For example, add 3 µl of the miRNeasy Serum/Plasma Spike-In Control \(the manufacturer's instructions produce a working solution of 1.6 x 108 copies/µl, so if you add 3 µl of the Spike-In Control and elute your exRNA in a final volume of 30 µl, the theoretical concentration of the Spike-In Control in the final exRNA sample would be 1.6 x 107 copies/µl). 3) The centrifuge must be above 20°C so that excessive precipitation does not occur. 4) After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur. 5) To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor \(e.g., if the rotor rotates clockwise, orient the lids counterclockwise). It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no

ethanol is carried over during RNA elution. 6) As little as 10 µl RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 µl RNase-free water, as the spin column membrane will not be sufficiently hydrated. The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase-free water results in a 12 µl eluate.

Acknowledgements

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

Supplementary Files

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

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