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Isolation of exosomal RNA from serum or plasma using the Qiagen ExoRNeasy Midi kit

Xandra O. Breakefield (breakefield@hms.harvard.edu)
Departments of Neurology and Radiology and Program in Neuroscience, Harvard Medical School, Massachusetts General Hospital, Boston, MA, USA

Saumya Das (sdas@mgh.harvard.edu)
Cardiovascular Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 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

Anil K. Sood (asood@mdanderson.org)
Departments of Gynecologic Oncology and Reproductive Medicine and of Cancer Biology and Center for RNA Interference and Non-Coding RNAs, The University of Texas MD Anderson Cancer Center (MDACC), Houston, TX, USA

Leonora Balaj
Department of Neurology and Program in Neuroscience, Harvard Medical School, Massachusetts General Hospital, Boston, MA, USA

Justyna Filant
Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center (MDACC), Houston, TX, 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

Bridget Simonson
Cardiovascular Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

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

Xuan Zhang
Department of Neurology, Harvard Medical School, Massachusetts General Hospital, Boston, MA, USA

Louise C. Laurent

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

Method Article

Keywords: exRNA, extracellular vesicles, exosomes, serum, plasma, blood, ExoRNeasy

Posted Date: June 16th, 2017

DOI: https://doi.org/10.1038/protex.2017.073

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Abstract

This protocol describes how to isolate exosomal RNA from serum/plasma using the Qiagen ExoRNeasy kit. It supercedes an earlier protocol \(http://dx.doi.org/10.1038/protex.2015.112). 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

ExoRNeasy Midi kit \(Qiagen, 77044) RNeasy MinElute Columns \(Qiagen, Part of 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 Vortexer Phase lock gel tubes, 2 mL \(VWR, 10847-802)

Procedure

1. Transfer 500 µL of serum or plasma equilibrated to room temperature into a 1.5 mL microfuge tube. 2. Add 500 µL XBP stored at room temperature. 3. Invert 5 times. 4. Add 800 µL of sample/XBP mixture onto the exoEasy spin column and centrifuge for 1 minute at 500 x g at room temperature. 5. Repeat step 4 until all mixture is added onto the column. 6. Discard flow-through. 7. Add 800 µL XWP to the exoEasy spin column. 8. Centrifuge for 5 minutes at 5,000 x g 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 x g at room temperature. 12. Transfer the flow-though, which is the lysate, to a PLG tube.[1] 13. Vortex for 5 seconds. 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 21, including any precipitate that may have formed, onto the column. 24. Centrifuge for 15 seconds at 1,000 x g at room temperature.[2] 25. Discard flow-through. 26. Repeat steps 23-25 until entire sample has been loaded. 27. Make sure that ethanol has been added to RWT and RPE buffers. 28. Add 700 µL Buffer RWT to the RNeasy MinElute spin column. 29. Centrifuge for 15 seconds at ≥ 8000 x g at room temperature to wash the column. 30. Discard the flow-through. 31. Pipet 500 µL Buffer RPE onto the RNeasy MinElute spin column. 32. Centrifuge for 15 seconds at ≥ 8000 x g to wash the column. 33. Discard the flow-through. 34. Pipet 500 µL Buffer RPE onto the RNeasy MinElute spin column. 35. Centrifuge for 2 minutes at ≥ 8000 x g \(≥10,000 rpm) at room temperature to wash the column. 36. Discard the collection tube with the flow-through.[3] 37. Transfer the RNeasy MinElute spin column into a new 2 mLcollection tube \(supplied). 38. Open the lid of the spin column, and centrifuge at full speed \(14,000 x g) for 5 min to dry the membrane.[4] 39. Discard the collection tube with the flow-through. 40. Transfer the RNeasy MinElute spin column in a new 1.5 mL collection tube \(supplied). Add 30 µL RNase-free water directly to the center of the spin column membrane.[5] 41. Centrifuge for 1 min at full speed to elute the RNA \(Optional: Centrifuge for 1 min at 100 x g followed by 1 min at full speed).[6]

Troubleshooting

[1] PLG makes separation of aqueous phase from the interface easier, and thus is particularly useful for large numbers of samples or less experienced personnel, but it is expensive. [2] The manufacturer's protocol is for 8000 x g, but some labs have found that 1000 x g for the binding step gives better results. [3] 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. [4] 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. [5] This volume was selected to match that of other kits to enable fair comparisons. 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. [6] Centrifuging at a low speed first helps the solvent wet the surface of the membrane prior to the full-speed centrifuging step. This results in a better yield/RNA recovery from the membrane.

Acknowledgements

This protocol was modified from the Qiagen ExoRNeasy midi kit. JF and AS were supported by NIH grant UH3 TR000943. PN, AP and RG were supported by UH2-UH3 TR000890. BS and SD were supported by

UH3TR000901. SS and LL were supported by U01 HL126494 and UH3 TR000906. XZ, LB and XOB were supported by U19 CA179463.