Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Isolation of exosomal RNA from serum or plasma using the Norgen BioTek Plasma/Serum circulating and exosomal RNA purification mini kit

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

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

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

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, Norgen BioTek

Posted Date: June 16th, 2017

DOI: https://doi.org/10.1038/protex.2017.078

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

This protocol describes how to isolate total extracellular RNA from serum or plasma using the Norgen BioTek Plasma/Serum circulating and exosomal RNA purification mini kit. It supercedes an earlier protocol \(http://dx.doi.org/10.1038/protex.2015.116). 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

Plasma/Serum circulating and exosomal RNA purification mini kit \(Norgen BioTek – 51000) 2-Mercaptoethanol, 99% Extra Pure \(Acros Organics – 125472500) 100% ethanol \(Koptec – V1016) RNase-free water \(Ambion – AM9937)

Equipment

Amicon Ultra 0.5 mL Centrifugal filter devices – 3K \(Amicon – UFC500396) Microfuge 1.5 mL Microfuge tubes Vortexer

Procedure

1. Set Incubator to 60°C and warm PS Solution A, PS Solution B, and PS Solution C for 20 minutes to ensure no precipitates are present. 2. Add 50 mL of 96-100% ethanol to Wash Solution and check off box on bottle noting addition of ethanol. 3. Add 10 µL 2-Mercaptoethanol per 1 mL PS Solution B.[1] 4. Transfer 500 µL biofluid into a microfuge tube. 5. Add 100 µL PS Solution A[2] and 900 µL PS Solution B \(containing 2-Mercaptoethanol). 6. Vortex for 15 seconds. 7. Incubate for 10 min at 60°C. 8. Add 1.5 mL 100% Ethanol. 9. Vortex for 15 seconds. 10. Centrifuge for 30 seconds at 100 x g at room temperature. Carefully decant supernatant, discarding it. DO NOT disrupt pellet. 11. To the pellet add 750 µL PS Solution C. 12. Vortex for 15 seconds. 13. Incubate for 10 min at 60°C. 14. Add 750 µL 100% Ethanol. 15. Vortex for 15 seconds. 16. Assemble a mini filter Spin Column with a collection tube. 17. Transfer 650 µL of the mixture to the Filter Column. 18. Centrifuge for 1 min at 16,000 x g at room temperature. 19.

Discard flow-through. 20. Repeat steps 17-19 until all of the mixture has been added to the Filter Column. 21. Add 400 µL Wash Solution to the column. 22. Centrifuge for 1 min at 16,000 x g at room temperature. 23. Discard flow-through. 24. Repeat steps 21-23 two more times for a total of 3 washes. 25. Open the cap of the column and centrifuge for 3 minutes at 16,000 x g at room temperature to dry the column. Discard collection tube. 26. Transfer the spin column to a fresh 1.7 mL elution tube. 27. Apply 30 µL water to the column.[3] 28. Centrifuge for 2 mins at 300 x g at room temperature to load the solution onto the column. 29. Centrifuge for 3 min at 16,000 x g at room temperature to elute the RNA.[4]

Troubleshooting

[1] If the kit will be used up within 3 months, the 2-Mercaptoethanol can be added directly to the Solution B bottle. [2] Solution A contains a resin and must be mixed well before addition to samples. [3] The manufacturer's protocol suggests eluting with 100 µL of Elution Solution A. This volume was selected to match that of other kits to enable fair comparisons. Eluting with larger volumes will lead to a better yield but more dilute samples. [4] Optional clean-up by Amicon filtration 1. Dilute the eluted RNA with 320 µL DNA / RNA free water. 2. Place the Amicon column into a collection tube. Load sample onto column. 3. Spin for 8 minutes at 14,000 x g at room temperature until the volume of the sample is ~20 µL. 4. Discard the flow through. 5. Invert the colum and place it into a new tube. 6. Centrifuge for 2 minutes at 8,000 x g at room temperature. 7. The final volume should be ~20 µL. The sample can be further concentrated using a speed-vac.

Acknowledgements

This protocol was modified from the manufacturer's instructions for the Norgen BioTek Plasma/Serum circulating and exosomal RNA purification mini kit. JF and AS were supported by NIH grant UH3 TR000943. PN, AP and RG were supported by UH2-UH3 TR000890. SS and LL were supported by U01 HL126494 and UH3 TR000906.