RNA isolation from human serum and plasma samples using the Norgen exosomal RNA purification mini kit

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Method Article

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Abstract

This protocol describes how to isolate RNA from human serum and plasma using the Norgen exosomal RNA purification mini kit in order to detect, identify and quantify extracellular RNA.

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 RNA isolation from human serum and plasma using the Norgen exosomal RNA purification mini kit is one of the RNA isolation methods compared in "the associated publication":http://www.journalofextracellularvesicles.net/index.php/jev/article/view/26533.

Reagents

Plasma/Serum Circulating and Exosomal RNA Purification Mini Kit \(Slurry Format) \(Norgen Biotek, catalog # 51000) 2-mercaptoethanol, 99% Extra Pure \(ACROS ORGANICS, catalog # 125472500) ethanol

Equipment

Microfuge Amicon Ultra 0.5 mL Centrifugal Filter Devices - 3K \(Amicon, catalog # UFC500396)

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 200 µl biofluid into a microfuge tube. 5. Add 40 µl PS Solution A[2] and 360 µl PS Solution B \(containing 2-Mercaptoethanol). 6. Vortex for 15 seconds. 7. Incubate for 10 min at 60°C. 8. Add 600 µl 100%

Ethanol. 9. Vortex for 15 seconds. 10. Centrifuge for 30 seconds at 1,000 rpm at room temperature. Carefully decant supernatant, discarding it. DO NOT disrupt pellet. 11. To the pellet add 300 µl PS Solution C. 12. Vortex for 15 seconds. 13. Incubate for 10 min at 60°C. 14. Add 300 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 14,000 rpm 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 14,000 rpm 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 14,000 rpm 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 100 µL Elution Solution to the column. 28. Centrifuge for 2 mins at 2,000 rpm at room temperature to load the solution onto the column. 28. Centrifuge for 3 min at 14,000 rpm at room temperature to elute the RNA.

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) Optional clean-up by Amicon filtration 1. Dilute the eluted RNA with 320µL 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 xg at room temperature until the volume of the same is ~20 µl. 4. Discard the flow through. 5. Invert the column and place it into a new tube. 6. Centrifuge for 2 minutes at 8,000 xg 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.