Isolation of exosomal RNA from urine using the Qiagen ExoRNeasy maxi kit

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Method Article

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Abstract

This protocol describes how to isolate exosomal RNA from urine by using the Qiagen ExoRNeasy maxi kit.

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 Maxi kit \(Qiagen, 77064) RNeasy MinElute Columns \(Qiagen, part of miRNeasy micro kit - 217084) Urine \(pre-cleared by centrifugation at 2000 x g for 10 min) Chloroform \(Sigma-Aldrich, 319988) 100% ethanol \(Koptec, V1016) RNase-free water \(Ambion, AM9937)

Equipment

Phase lock gel tubes, 2 mL \(VWR, 10847-802) Microfuge Centrifuge Microfuge tubes, 1.5 mL

Procedure

1. Thaw samples on ice 2. Transfer 0.5 mL of urine into a 1.5 mL microfuge tube. 2. Add 0.5 mL XBP. 3. Invert 5 times. 4. Add the sample/XBP mixture onto the exoEasy spin column \(in 15 mL collection tube). 5. Centrifuge for 1 min at 500 x g at room temperature. 6. Discard flow-through. 7. Add 3.5 mL XWP to the exoEasy spin column. 8. Centrifuge for 5 min 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 min 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 s.[2] 14. Incubate at room temperature for 5 min. 15. Add 90 µL chloroform. 16. Shake vigorously for 15 s. 17. Incubate for 3 min at room temperature. 18. Centrifuge sample for 15 min at 12,000 x g at 4°C. After this spin, allow centrifuge to warm up to room temperature. 19. Transfer the upper aqueous phase \(270 µL) to a new microcentrifuge tube. 20. Carefully measure the aqueous phase and add 2 volumes \(540 µL) 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 s 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 Buffer RWT to the RNeasy MinElute spin column. 28. Centrifuge for 15 s 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 s 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 min 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 x g) 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 to 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 min at 100 x g. 41. Centrifuge at full speed for 1 min to elute the RNA.

Troubleshooting

[1] PLG makes separation of the 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] 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.

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