Isolation of exosomal RNA from cell culture supernatant 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 cell culture supernatant 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) Chloroform \(Sigma-Aldrich, 319988-500 mL) 100% ethanol \(VWR, 89125-186)

Equipment

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

Procedure

1. Harvest cell culture medium from 80% confluent cells grown in serum-free medium. 2. Optional: Spin media at 500-1,000 x g for 10 min. 3. Pre-clear medium by centrifuging at 2000 x g for 10 min then filter using a 0.22 µm PES Steriflip membrane and freeze at -80°C until needed. 4. Thaw 4 mL of cell culture supernatant on ice. 5. Transfer 4 mL of cell culture supernatant to a 15 mL tube and add 4 mL of XBP buffer. 6. Mix by inverting 5 times. 7. Add the sample/XBP mix to the exoEasy maxi spin column. 8. Centrifuge for 1 – 3 min at 500 x g at room temperature and discard flow-through. 9. Add 10 mL of XWP to the spin column and centrifuge for 5 min at 5,000 x g[1] at room temperature. 10. Transfer the spin column to a fresh collection tube. 11. Add 700 µL of Qiazol to the spin column and centrifuge for 5 min at 5,000 x g.[1] 12. Spin PLG tubes for 30 s at 16,000 x g. 13. Add flow-through from spin column in step 11 to PLG tube, and vortex for 5 s.[2] 14. Incubate at room temperature for 5 min. 15. Add 90 µL of chloroform, then shake \(do not vortex) vigorously for 15 s. 16. Incubate for 2 – 3 min at room temperature. 17. Centrifuge samples for 15 min at 12,000 x g at 4°C and transfer the upper aqueous phase to a new microcentrifuge tube. 18. Measure the aqueous phase and add 2 volumes 100% ethanol. 19. Mix gently and thoroughly by pipetting up and down. 20. Immediately add up to 700 µL of the mix from step 19, including any precipitate, onto the MinElute spin column. 21. Centrifuge for 15 s at 1,000 x g at room temperature and discard the flow-through.[3] 22. Repeat steps 20-21 until all the sample has been added. 23. Make sure ethanol has been added to the RWT and RPE buffers as recommended by the

manufacturer. 24. Add 700 µL of Buffer RWT to the spin column and centrifuge for 15 s at ≥8,000 x g at room temperature to wash the column and discard the flow-through.[4] 25. Add 500 µL of Buffer RPE onto the column and centrifuge for 15 s at ≥8,000 x g at room temperature and discard the flow-through. 26. Add 500 µL of Buffer RPE onto the column and centrifuge for 2 min at ≥8,000 x g at room temperature and discard the flow-through. 27. Transfer the spin column to a new 2 mL collection tube. 28. Open the lid of the column and centrifuge at full speed for 5 min to dry the membrane.[5] 29. Discard the collection tube and flow-through. 30. Transfer the spin column to a new 1.5 mL collection tube, add 14 µL or 30 µL of RNase-free water directly to the center of the spin column.[6] 31. Centrifuge for 1 min at 100 x g.[7] 32. Centrifuge for 1 min at full speed to elute RNA.

Troubleshooting

[1] Spinning at 3,500 x g is acceptable if a 5,000 x g spin rate is not available on your tabletop centrifuge. [2] 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. [3] 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. [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] 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. [7] 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.

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