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Isolation of exosomal RNA from urine using the Qiagen miRNeasy Micro kit

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Method Article

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Abstract

This protocol describes how to isolate total extracellular RNA from urine using the Qiagen miRNeasy Micro kit. It supercedes an earlier protocol \(http://dx.doi.org/10.1038/protex.2015.115). 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

miRNeasy micro kit \(Qiagen, 217084) Chloroform \(Sigma-Aldrich, 319988) 100% ethanol \(Koptec, V1016) 80% ethanol RNase-free water \(Ambion, AM9937)

Equipment

Refrigerated microfuge or tabletop centrifuge 15 mL Centrifuge tubes 1.5 mL Microfuge tubes Vortexer Phase lock gel tubes \(PLG), 2 mL \(VWR, 10847-802)

Procedure

1. Thaw samples on ice. 2. Transfer 500 µL of urine into a 15 mL centrifuge tube. If using the exosomal fraction from ultracentrifugation or Millipore methods, transfer 200 µL. 3. Add 5x volumes of QIAzol Lysis Reagent.[1] 4. Vortex 5 s. 5. Incubate for 5 min at room temperature. 6. Add 1x volume chloroform. 7. Shake \(do not vortex) vigorously for 15 s. 8. Incubate for 3 min at room temperature. 9. Centrifuge sample for 15 min at 12,000 x g at 4°C. 10. Transfer the upper aqueous phase to a new 15 mL centrifuge tube. 11. Carefully measure the aqueous phase and add 1.5x volumes of 100% ethanol. 12. Mix gently and thoroughly. Do not centrifuge and do not delay moving on to the next step. 13. Assemble a MinElute spin column in a new collection tube. 14. Load up to 700 µL of the mixture, including any precipitate that may have formed, onto the column. 15. Spin for 15 s at 1,000 x g^2 at room temperature.[3] 16. Discard flow-through. 17. Repeat steps 14-16 until entire sample has been loaded. 18. Add 700 µL Buffer RWT to the RNeasy MinElute spin column. 19. Centrifuge for 15 s at ≥ 8000 x g at room temperature to wash the

column. 20. Discard the flow-through. 21. Pipet 500 µL Buffer RPE onto the RNeasy MinElute spin column. 22. Centrifuge for 15 s at ≥ 8000 x g to wash the column. 23. Discard the flow-through. 24. Pipet 500 µL fresh 80% ethanol[4] onto the RNeasy MinElute spin column. 25. Centrifuge for 2 min at ≥ 8000 x g \(≥10,000 rpm) at room temperature to wash the column.[5] 26. Discard the collection tube with the flow-through. 27. Transfer the RNeasy MinElute spin column into a new 2 mL collection tube \(supplied). 28. Open the lid of the spin column, and centrifuge at full speed for 5 min to dry the membrane.[6] 29. Discard the collection tube with the flow-through. 30. Transfer the RNeasy MinElute spin column into a new 1.5 mL collection tube \(supplied). Add 14 µL RNase-free water directly to the center of the spin column membrane.[7] 31. Centrifuge for 1 min at 100 x g followed by 1 min at full speed to elute the RNA. 32. Aliquot 5µL into new microcentrifuge tube for QC analyses.

Troubleshooting

[1] Adapted from miRNeasy serum/plasma kit – added 5x volume of Qiazol [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] The centrifuge must be above 20°C so that excessive precipitation does not occur. [4] 80% ethanol should be prepared with ethanol \(96–100%) and RNase-free water. [5] 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. [6] 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. [7] 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.

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