

RNA isolation using the miRNeasy Micro Kit

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Abstract

This protocol describes how to isolate RNA from biofluids using the miRNeasy Micro 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/>). This protocol for RNA isolation from biofluids using the miRNeasy Micro Kit is one of the RNA isolation methods compared in "the associated publication": <http://www.journalofextracellularvesicles.net/index.php/jev/article/view/26533>.

Reagents

miRNeasy Micro Kit (Qiagen, catalog # 217084) chloroform 70% Ethanol 100% Ethanol RNase-free water

Equipment

Microfuge Microfuge tubes, 1.5 ml

Procedure

1. Transfer 200 µl of biofluid into a 1.5 ml microfuge tube.
2. Add 1000 µl (5x volumes) of QIAzol Lysis Reagent.
3. Vortex 5 sec.
4. Incubate for 5 min at room temperature. (2,3)
5. Add 200 µl chloroform.
6. Shake vigorously for 15 seconds.
7. Incubate for 3 minutes at room temperature.
8. Centrifuge sample for 15 min at 12,000 x g at 4°C.
9. Transfer the upper aqueous phase to a new microcentrifuge tube (4).
10. Avoid the white interphase. Carefully measure the aqueous phase and add 1.5 volumes of 100% ethanol.
11. Mix gently and thoroughly. Do not centrifuge and do not delay moving on to the next step.

12. Assemble a MinElute spin column in a new collection tube. 13. Load up to 700 μl of the mixture from step 5, including any precipitate that may have formed, onto the column. 14. Spin for 15 seconds at $8,000 \times g$ at room temperature. 15. Discard flow-through. 16. Repeat steps 13-15 until the entire sample has been loaded. 17. Add 700 μl Buffer RWT to the RNeasy MinElute spin column. 18. Centrifuge for 15 seconds at $8000 \times g$ at room temperature to wash the column. 19. Discard the flow-through. 20. Pipet 500 μl Buffer RPE onto the RNeasy MinElute spin column. 21. Centrifuge for 15 seconds at $8000 \times g$ to wash the column. 22. Discard the flow-through. 23. Pipet 500 μl 80% ethanol onto the RNeasy MinElute spin column. 24. Centrifuge for 2 minutes at $\geq 8000 \times g$ ($\geq 10,000 \text{ rpm}$) at room temperature to wash the column. 25. Discard the collection tube with the flow-through. 26. Transfer the RNeasy MinElute spin column into a new 2 ml collection tube (supplied). 27. Open the lid of the spin column, and centrifuge at full speed for 5 min to dry the membrane. 28. Discard the collection tube with the flow-through. 29. 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. 30. Centrifuge for 1 minute at full speed to elute the RNA.

Troubleshooting

(1) The only difference between the micro kit and the serum/plasma kit is the Ce_miR-39 Assay. (2) Trizol LS is “slightly” more concentrated than standard Trizol or Qiazol, according to a tech note from Life Technologies, allowing a reduction in the ratio of lysis reagent:biofluid. According to the tech note, a ratio of 10:1 Trizol:biofluid can be adjusted to a ratio of 3:1 Trizol LS:biofluid. This may allow processing of larger volumes at one time, but different ratios of these reagent:biofluid have not been rigorously tested. (3) 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 1.6×10^8 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 1.6×10^7 copies/ μl). (4) If there is poor phase separation, the original biofluid can be diluted. To “rescue” the exRNA preparation, additional buffer, Qiazol and chloroform can be added (maintaining the ratio 1:5:1). (5) The manufacturer’s protocol is for $8000 \times g$, but some labs have found that $1000 \times g$ for the binding step gives better results. (6) The centrifuge must be above 20°C so that excessive precipitation does not occur. (7) 80% ethanol should be prepared with ethanol (96–100%) and RNase-free water. (8) 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. (9) 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. (10) 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 μ l: elution with 14 μ l RNase-free water results in a 12 μ l eluate.

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This protocol was modified from the manufacturer's instructions for the miRNeasy Micro Kit.