# Isolation of extracellular vesicles and RNA using the miRcury Biofluids Kit

**Louise C. Laurent** ( llaurent@ucsd.edu )
University of California San Diego, Sanford Consortium for Regenerative Medicine, 2880 Torrey Pines Scenic Drive, La Jolla, CA

**Roger P. Alexander** ( rogerpalexander@gmail.com )
Sample and Assay Standards Working Group of the Extracellular RNA Communication Consortium

**Method Article**

**Keywords:** exRNA, miRcury

**Posted Date:** December 21st, 2015

**DOI:** https://doi.org/10.1038/protex.2015.114

# Abstract

This protocol describes how to isolate extracellular vesicles \(EVs) and extracellular RNA \(exRNA) from biofluids using the miRcury Biofluids Kit in order to detect, identify and quantify exRNA. ---------- COMMENTS Title and abstract modified. - Ashleigh Carver, Editorial Assistant, Nature Protocols, 14/09/2016

# 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 isolation of EVs and exRNA from biofluids using the miRcury Biofluids Kit is one of the EV / RNA isolation methods compared in the "associated publication.":http://www.journalofextracellularvesicles.net/index.php/jev/article/view/26533.

# Reagents

miRcury Biofluids Kit \(Exiqon, catalog # 300112/300113) isopropanol 100% ethanol RNase-free water

# Equipment

Microfuge Microfuge tubes, 1.5 mL

# Procedure

1. Add 80 mL of 100% ethanol to **Wash Solution 2 BF**. 2. Prepare rDNase by adding 3 mL **Reaction Buffer for rDNase** to the rDNase vial. Incubate at room temperature for 1 minute. Swirl gently to dissolve – do NOT vortex. Aliquot and store at -20°C. 3. Transfer 200 µl of biofluid into a 1.5 ml microfuge tube. 4. Add 60 µl **Lysis Solution BF**. 5. Vortex for 5 seconds. 6. Incubate for 3 minutes at room

temperature. 7. Add 20 µl of **Protein Precipitation Solution BF**. 8. Vortex for 5 seconds. 9. Incubate for 1 minute at room temperature. 10. Centrifuge sample for 3 min at 11,000 x g at room temperature. 11. Transfer the clear supernatant to a new microcentrifuge tube. 12. Add 270 µl isopropanol. 13. Vortex for 5 seconds. 14. Assemble the **microRNA Spin Column BF** and load sample onto column. 15. Incubate 2 minutes at room temperature. 16. Spin for 30 seconds at 11,000 x g. Discard flow-through. 17. Add 700 µl **Wash Solution 2 BF**. 18. Spin for 30 seconds at 11,000 x g. Discard flow-through. 19. Add 250 µl **Wash Solution 2 BF**. 20. Spin for 2 minutes at 11,000 x g. 21. Add 50 µl rDNAse directly onto the membrane of the spin column. 22. Incubate for 15 minutes at room temperature. 23. Add 100 µl **Wash Solution 1 BF**. 24. Spin for 30 seconds at 11,000 x g. Discard flow-through. 25. Add 700 µl **Wash Solution 2 BF**. 26. Spin for 30 seconds at 11,000 x g. Discard flow-through. 27. Add 250 µl **Wash Solution 2 BF**. 28. Spin for 2 minutes at 11,000 x g. Transfer spin column to fresh microfuge tube. 29. Add 50 µl water directly onto the membrane of the spin column. 30. Incubate for 1 minute at room temperature. 31. Spin for 1 minute at 11,000 x g.

## Acknowledgements

This protocol was modified from the manufacturer’s instructions for the miRcury Biofluids Kit.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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