# Isolation of extracellular RNA from urine using Millipore membrane filtration

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**Method Article**

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## Abstract

This protocol describes how to isolate exosomal RNA from urine by using Millipore membrane filters for exosomal enrichment followed by the Qiagen miRNeasy Micro kit for RNA isolation.


## 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

Urine \(pre-cleared by centrifugation at 2000 x g for 10 min) Phosphate-buffered saline \(PBS), pH 7.4

## Equipment

AU-0.5 filter, 0.5 mL, 10 kDa MWCO \(EMD Millipore, UFC501024) Microcentrifuge

## Procedure

1. Thaw samples on ice. 2. Add 500 µL PBS to the AU-0.5 filter, cap, and centrifuge for 10 minutes at 14,000 x g. 3. Reverse the device and centrifuge for 2 minutes at 1000 x g to remove the residual PBS. 4. Aspirate PBS from the collection tube. 5. Transfer 500 µL of sample to the AU-0.5 filter and cap the device. 6. Centrifuge for 30 minutes at 14,000 x g. There should be ~15 µL sample remaining in the upper chamber. 7. Remove device from the centrifuge and empty the collection tube. 8. Add 500 µL PBS to the filter device and gently pipette sample multiple times to mix. 9. Centrifuge for 30 minutes at 14,000 x g. 10. Place filter upside down in a fresh microcentrifuge tube. 11. Centrifuge for 2 minutes at 2000 x g[1] to transfer the sample to the tube. 12. Isolate RNA using the miRNeasy Micro kit.

## Troubleshooting

[1] The manufacturer’s protocol suggests spinning at 1000 x g.

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