

# Urine Collection Procedure for the analysis of extracellular RNA

Louise C. Laurent (✉ [lolaurent@ucsd.edu](mailto:lolaurent@ucsd.edu))

University of California San Diego, Sanford Consortium for Regenerative Medicine, 2880 Torrey Pines Scenic Drive, La Jolla, CA

Roger Alexander

Sample and Assay Standards Working Group of the Extracellular RNA Communication Consortium

---

## Method Article

**Keywords:** exRNA, sample collection, urine

**Posted Date:** December 7th, 2015

**DOI:** <https://doi.org/10.1038/protex.2015.099>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

This protocol describes how to collect urine samples 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 collecting urine is one of the biofluid collection and processing methods compared in "the associated publication": <http://www.journalofextracellularvesicles.net/index.php/jev/article/view/26533>.

## Equipment

urine collection cup, sterile: Vendor: Fisher, Catalog # 22-610-131 Falcon™ 15mL Conical Centrifuge Tubes, polypropylene: Vendor: Fisher, Catalog # 14-959-70C Falcon™ 50mL Conical Centrifuge Tubes, polypropylene: Vendor: Fisher, Catalog # 14-432-22 Sterile Microcentrifuge Tubes with Screw Caps: Vendor: Fisher, Catalog # 2681375 Avantec syringe filters, 25 mm, 0.8 µm cellulose acetate filter: Vendor: Cole Parmer, Catalog # EW-81054-40 Table 1: Equipment list in tabular format for urine collection procedure [See figure in Figures section](#).

## Procedure

1. Sample should be processed, aliquoted and placed into the freezer within 2 hours of collection.
2. Collect approximately 50 ml urine in an empty sterile urine collection cup as a midstream sample.
3. Transfer urine to a 50 ml centrifuge tube.
4. Centrifuge tubes at 500 xg for 10 minutes at room temperature.
5. Transfer the urine to a fresh 50 ml centrifuge tube. Avoid any material at the bottom of the tube.
6. Centrifuge tubes at 2000 xg for 10 minutes at room temperature.
7. Transfer the top 80% of the urine, avoiding any material at the bottom of the tube, to fresh 15 ml tubes, dispensing at most 10 ml

urine per tube \ (should be 4 tubes total).<sup>1</sup> 8. Using a sterile pipet, make 3 aliquots of 1 ml each in screw-cap microcentrifuge tubes.<sup>2</sup> 9. Place the four 15 ml centrifuge tubes and three 1 ml aliquots of urine into a -80°C freezer for storage.

## Troubleshooting

1) As an additional step to remove residual cells, the urine may be passed through a 0.8 µm cellulose acetate filter. This step should be performed slowly to avoid shear damage to cells or extracellular vesicles. This step may deplete the urine of larger vesicles. 2) These aliquots may be used for quality control.

## Figures

Item	Vendor	Catalog #
urine collection cup, sterile	Fisher	22-610-131
Falcon™ 15mL Conical Centrifuge Tubes, polypropylene	Fisher	14-959-70C
Falcon™ 50mL Conical Centrifuge Tubes, polypropylene	Fisher	14-432-22
Sterile Microcentrifuge Tubes with Screw Caps	Fisher	2681375
Avantec syringe filters, 25 mm, 0.8 um cellulose acetate filter	Cole Parmer	EW-81054-40

### Figure 1

Table 1 Equipment list in tabular format for Urine Collection Procedure