

# Serum Collection Procedure (Small Scale) for the analysis of extracellular RNA

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## Method Article

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# Abstract

This protocol describes how to collect small-scale serum 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 serum 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

Butterfly Blood Collection Set, 21g: Vendor: Becton-Dickson, Catalog # 367287 Vacutainer Holder: Vendor: Becton-Dickson, Catalog # 364815 Serum tube, 10mL: Vendor: Becton-Dickson, Catalog # 367820 Latex-Free Tourniquet: Vendor: Medicus Health, Catalog # 5343M1 Falcon™ 15mL Conical Centrifuge Tubes, polypropylene: Vendor: Fisher, Catalog # 14-959-70C Sterile Microcentrifuge Tubes with Screw Caps: Vendor: Fisher, Catalog # 2681375 Table 1: Equipment list in tabular format [See figure in Figures section](#).

## Procedure

1. Sample should be processed, aliquoted and placed into the freezer within 2 hours of collection.
2. Collect 10 ml whole blood by peripheral venipuncture using a 21 gauge butterfly needle into a serum vacutainer tube.
3. Store tube upright for 10-30 minutes at room temperature<sup>1</sup>, to allow blood to clot.
4. Centrifuge tubes at 500 xg for 10 minutes at room temperature.
5. Transfer the serum to a fresh 15 ml centrifuge tube.
6. Avoid the red cell layer (the dark layer at the bottom).
6. Centrifuge tubes at 2000 xg for 10 minutes at room temperature.
7. Using a fresh pipet, transfer the top 80% of the serum, avoiding any

material at the bottom of the tube, to a fresh 15 ml tube. 8. Transfer 1 ml of serum into each screw-cap microcentrifuge tube. 9. Place the 1 ml aliquots into a -80°C freezer for storage.

## Troubleshooting

1) If the blood is put at 4°C, the serum will become cloudy and the serum and red cells will not separate well during centrifugation. 2) As an additional step to remove residual cells, the serum 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 serum of larger vesicles, such as oncosomes.

## Figures

<b>Item</b>	<b>Vendor</b>	<b>Catalog #</b>
Butterfly Blood Collection set, 21g	Becton-Dickinson	367287
Vacutainer Holder	Becton-Dickinson	364815
Serum tube, 10 ml	Becton-Dickinson	367820
Latex-Free Tourniquet	Medicus Health	5343M1
Falcon™ 15mL Conical Centrifuge Tubes, polypropylene	Fisher	14-959-70C
Sterile Microcentrifuge Tubes with Screw Caps	Fisher	2681375

Figure 1

Table 1 Reagents for Serum Collection Procedure (small scale)