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SHORT PROTOCOL No. 63

# Collection of Exosomes from Blood Using Centrifuge CS-(F)NX Series

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## Introduction

Exosomes are secreted by cells into body fluids as membrane vesicles surrounded by a lipid bilayer and circulate throughout the body. Exosomes contain proteins, nucleic acids, lipids and metabolites within their vesicles and have been shown to act as intercellular communication media [1]. Recently, biomarkers have been discovered in exosomes that reflect the state of disease in the cells from which they are secreted; these have attracted attention as diagnostic markers [2]. Since exosomes are found in body fluids (blood, urine, saliva, etc.,) they are attracting attention as liquid biopsies. Liquid biopsies do not require direct sampling of the disease site (minimally invasive) and are less burdensome for patients [3] [4]. Ultracentrifugation is recommended for the purification of exosomes, using swing-bucket rotors. Exosomes can be collected at the bottom of the centrifuge tube as a precipitate and can therefore be recovered efficiently. However, a swing-bucket rotor cannot process many samples at once.

In this SHORT PROTOCOL, the fixed-angle rotor for 1.5 mL micro tube (C) was used to collect and purify exosomes. The 1.5 mL micro tube (C) has a conical bottom, which makes it easy for precipitates to collect at a single point at the bottom, even with a small sample volume. Fixed-angle rotors can process multiple samples at once and separate fractions in a shorter time than a swing-bucket rotor.


**Figure 1:** Centrifuge CS150NX and Centrifuge CS150FNX with Rotor S55A2

## Materials and Methods

### Materials used

Centrifuge CS150NX or Centrifuge CS150FNX with Rotor S55A2 for 1.5 mL micro tubes (C) was used. The Rotor S55A2 has a maximum speed of 55,000 rpm (201,000 x *g*). 12 tubes can be centrifuged simultaneously. The 1.5 mL micro tubes (C) are well suited for processing small sample volumes, especially as the 1.5 mL micro tubes (C) can be used with less than the specified volume and even empty. Accordingly, the workflow presented here can be performed with even smaller amounts of sample volume.

### Human serum purification process

Approximately 1 mL of human blood was collected and spun in a 1.5 mL microtube (Eppendorf Tubes 3810X) in Centrifuge 5425 R with Rotor FA-24x2 at 3,000 rpm (845 x *g*) for about 30 minutes. The serum supernatant was transferred to a new tube. The serum was then centrifuged at 10,319 rpm (10,000 x *g*) for 20-30 min at 4°C to remove cell debris. The supernatant was again transferred to a fresh tube.

### Exosome isolation process

1.5 mL micro tube (C) was placed inside the Rotor S55A2 in Centrifuge CS150NX or Centrifuge CS150FNX and centrifuged at 55,000 rpm (201,000 x *g*) for 20-30 min at 4°C. The supernatant was removed, and the pellet was resuspended in 1 mL PBS. The 1.5 mL micro tubes (C) was then placed into the Rotor S55A2 and centrifuged at 55,000 rpm (201,000 x *g*) for 20-30 min at 4°C. The supernatant was removed, and the pellet was resuspended in 50-100 µL PBS.

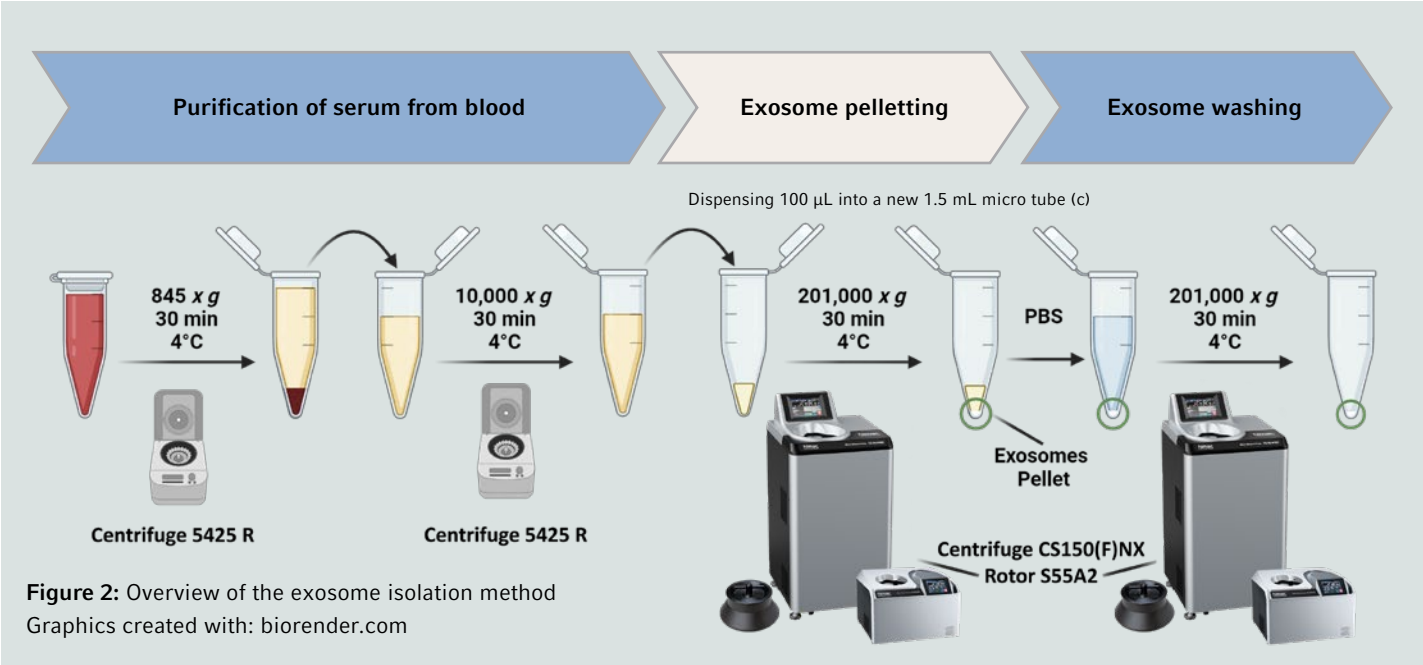


**Figure 2:** Overview of the exosome isolation method
Graphics created with: biorender.com

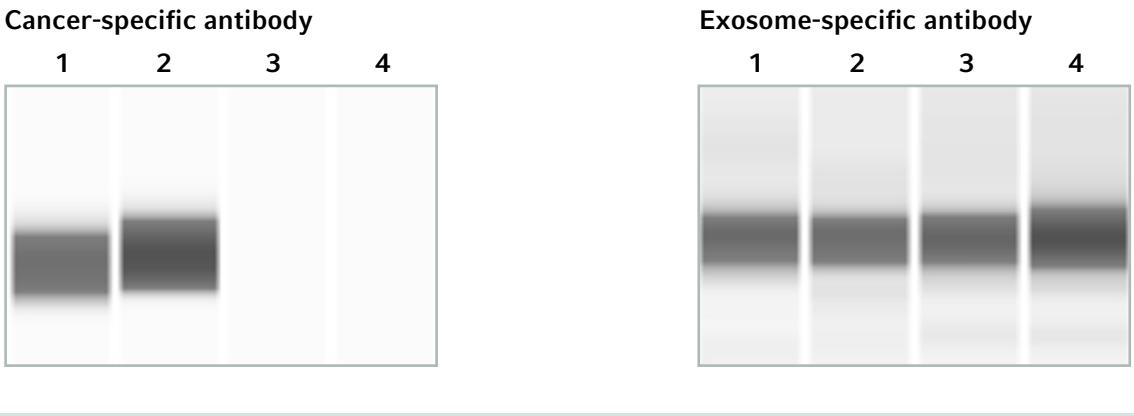


**Figure 3: Western blotting results**

Expression of a cancer marker on exosomes detected by western blotting. The cancer marker was detected only in cancer positive specimen. Lanes 1 and 2 contain positive specimen. Lanes 3 and 4 contain negative specimen.

Cancer marker (left panel) and exosome surface marker (right panel) detected by western blotting. Automated western blotting was performed on Simple Western (ProteinSimple, USA).

## Conclusion

In this SHORT PROTOCOL, the Centrifuge CS150NX and/or Centrifuge CS150FNX and Rotor S55A2 are used to isolate exosomes from human serum and detect exosome marker and cancer marker by western blotting. Ultracentrifugation is one of the most widely used techniques for exosome isolation. The separation conditions for ultracentrifugation using a swing-bucket rotor are described in many articles, but this is not always optimal for small sample volumes.

The 1.5 mL micro tubes (C) with its conical bottom and optional volume are ideal for pelleting small volumes of exosomes. In combination with the Rotor S55A2, allowing the centrifugation of 12 tubes at the same time, a high throughput can be achieved. The Rotor S55A2 for 1.5 mL micro tubes (C) can process large number of samples in a short time and is therefore considered the rotor of choice for high throughput.

**Acknowledgement**
This SHORT PROTOCOL was supported by Dr. Aya Shoda of Theoria Science, Inc.. Theoria Science Inc. was established on 17 May 2012 based on the research results of Dr. Takahiro Ochiya and his colleagues at Tokyo Medical University, who are leading experts in exosome research. The purpose of the company is to promote research and development of testing technologies and drug discovery for the early detection of cancer targeting exosome.

## Literature


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[3] Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A. 2004 Sep 7;101(36):13368-73. doi: 10.1073/pnas.0403453101.

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## Ordering information

**Ordering information**

| Description | Manufacturer | Order no. |
|---|---|---|
| **Centrifuge CS150NX** | Eppendorf | 5720 131 511 |
| **Centrifuge CS150FNX** | Eppendorf | 5720 121 511 |
| **Rotor S55A2** | Eppendorf | 5720 221 011 |
| **1.5 mL micro tube (C)** | Eppendorf | 5720 411 009 |
| **Centrifuge 5425 R** | Eppendorf | 5405 000 364 |
| **Rotor FA-24x2** | Eppendorf | 5495 500 006 |
| **Eppendorf Tubes 3810X** | Eppendorf | 0030 125 150 |


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