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SHORT PROTOCOL No. 57

Using OptiPrep® Density Gradient with Centrifuge CP100NX for Isolation of Purer Exosomes

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Abstract

This short protocol describes how to isolate the pure exosome population by using Centrifuge CP100NX to generate a density gradient in 40PET tubes loaded in Rotor P32ST.

Introduction

Exosomes are nanoscale vesicles consisting of a lipid bilayer membrane, with diameters of approximately 30 to 150 nm. They exist in body fluids such as blood, saliva, urine, amniotic fluid, and in cell culture media [1,2]. They are released into the extracellular matrix after the fusion of an intracellular membrane with the cell membrane [3].
In recent years, it has been reported that exosomes contain and wrap various proteins and RNA (mRNA, miRNA), suggesting the potential role of intercellular communication [4]. Utilizing this property, development as a tool of biomarker and targeted therapeutics is actively underway. Density gradient ultracentrifugation is effective for the isolation of high purity, high quality exosomes [5].
In Application Note 476 [6], the one-step sucrose cushion technique, coupled with ultracentrifugation, allowed the isolation of a pure and homogeneous population of exosomes from stem cells cultured in single-use bioreactors. These exosomes can be further purified for downstream applications by employing a longer centrifugation time and density gradient. This short protocol was designed for this purpose.
This short protocol shows how to isolate the pure exosome population by using the OptiPrep® density gradient centrifugation method utilizing the Centrifuge CP100NX and the Rotor P32ST. The combination of the ultracentrifuge, the swing-out rotor and the density gradient will allow the isolation of large amounts of the pure exosomes in a 40PET tube.

Materials and methods

Crude exosomes in PBS used in this short protocol were isolated (Figure 1B) using the sucrose cushion technique described in Application Note 476 [6] (Figure 1A).

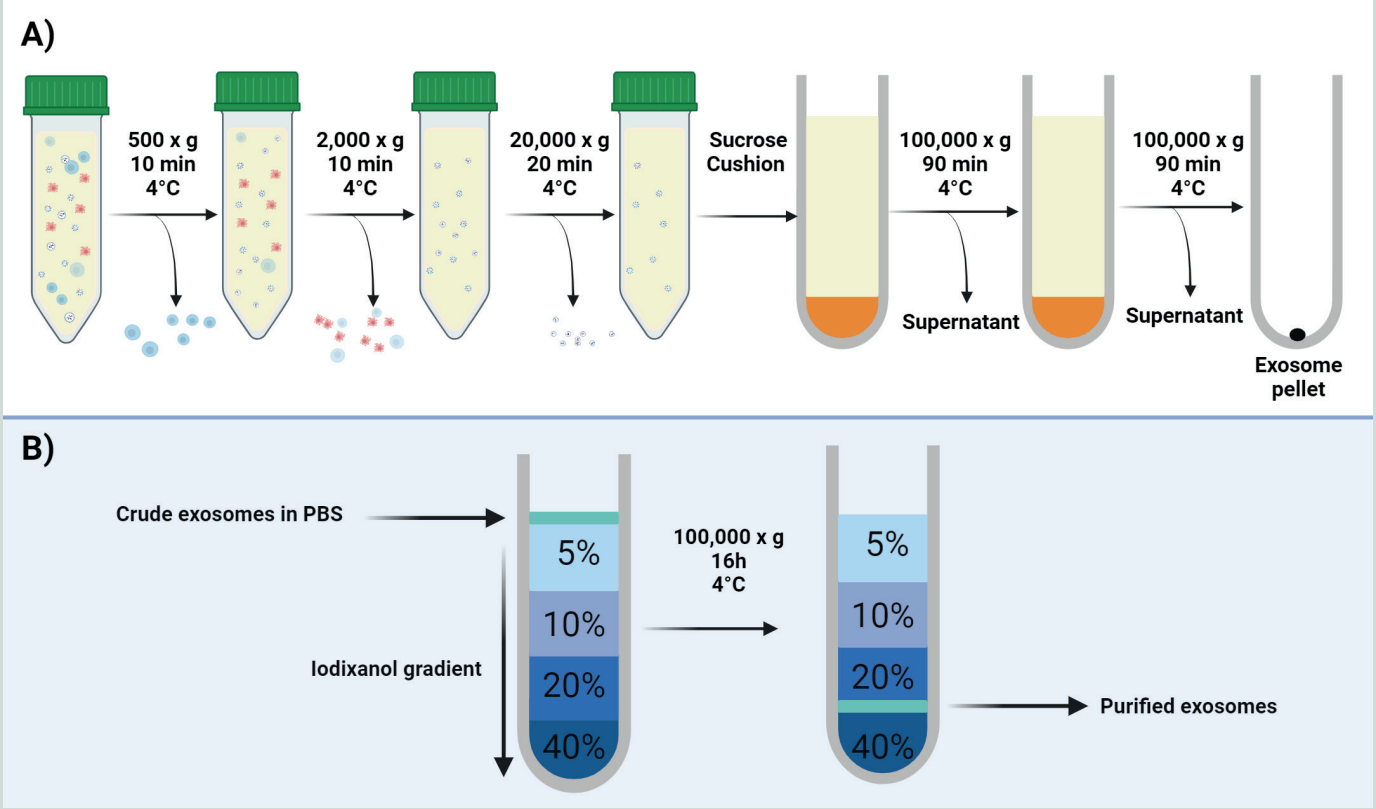

Figure 1 – Schematic representing the complete workflow for the isolation of exosomes with the first steps from Application Note 476 (A) and the purification of exosomes using the OptiPrep® (iodixanol) gradient technique (B) in the ultracentrifuge.
Image generated using Biorender.com

Equipment and reagents

> Centrifuge CP100NX
> Rotor P32ST
> 40PET tubes
> OptiPrep® (Iodixanol, AXS-1114542)
> Sucrose

Density gradient medium

Different concentrations of iodixanol were prepared using the stock solution of OptiPrep® 60 % iodixanol and dissolved in a sucrose solution. The density of the OptiPrep® is 1.32 g/cm^3. Sucrose was solubilized in a 30 nM Tris-HCl solution at a concentration of 0.25 M.

Four different gradient solutions (5, 10, 20, and 40 %) were prepared by mixing 60 % iodixanol with the 0.25 M sucrose solution. Layers were loaded in 40PET tubes as shown in figure 2A.

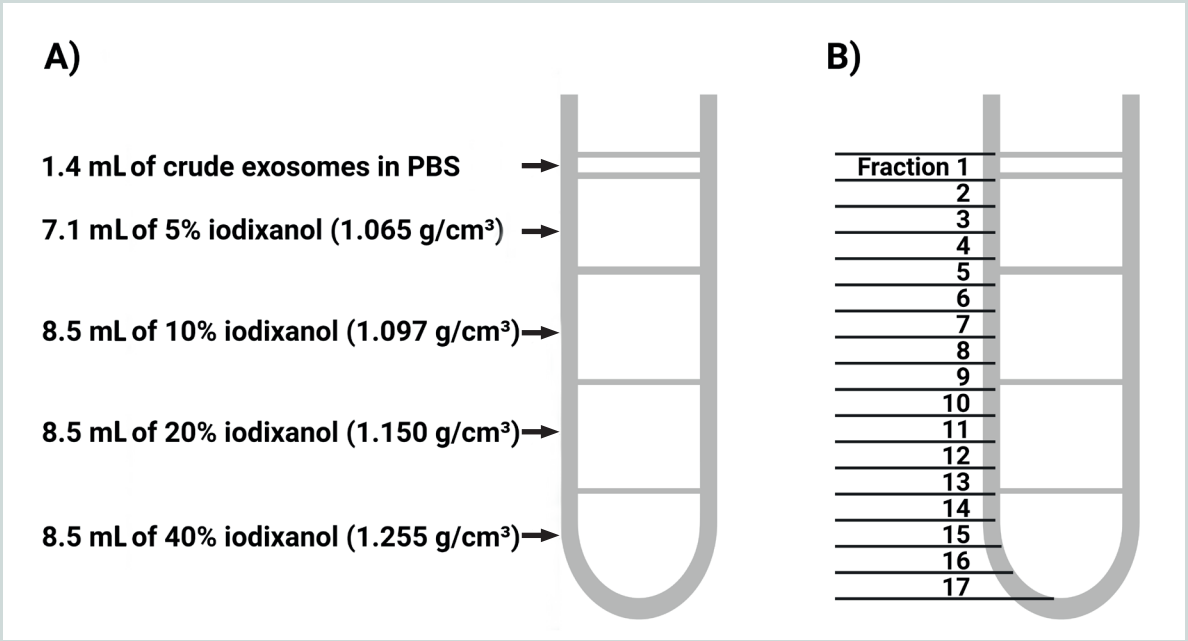

Figure 2 – Schematic representing the layers and composition of the density gradient before centrifugation (A). After centrifugation, fractions of 2 mL were collected from top to bottom (B). *Image generated using Biorender.com*

Exosome isolation

Crude exosomes in PBS, previously isolated using the sucrose cushion ultracentrifugation technique [6], were delicately positioned on top of the gradient solution in 40PET tubes (Figure 2A). Tubes were loaded into swing-out bucket Rotor P32ST and spun at 100,000 x g and 4 °C for 16 hours in Centrifuge CP100NX (Figure 3). Acceleration was set to 7 and deceleration was set to 0.

Exosome collection

After centrifugation, exosomes will be enriched in the density range of 1.15-1.19 g/cm^3, which can subsequently be collected by fractionation. Seventeen fractions of 2 mL were collected starting from the top (Figure 2B). The volume of each fraction was reduced to 300 µL using the Concentrator Plus for 135 min. Fractions were analyzed using density light scattering.

Figure 3 – Centrifuge CP100NX and Rotor P32ST.

Results and discussion

After centrifugation, exosomes were detected only in fraction 13 which represents the lowest part of the 20% iodixanol gradient. A single and narrow peak was detected by density light scattering at around 60 nm, which is the size of pure exosomes. Peaks representing background noise were detected in all other fractions (below 10 nm - Figure 4). Compared to other ultracentrifuge techniques, such as sucrose cushion [6], the density gradient allows for isolating pure exosomes using an ultracentrifuge. The sucrose cushion technique isolates a pure and homogeneous exosome population which is suitable for research projects. In the case of clinical research or therapy, exosome populations need to be in their purest form. Here, pure and homogeneous exosomes isolated from the sucrose technique were concentrated and cleaned with the OptiPrep® density gradient, represented by a single narrow peak at around 60 nm, showing no distortion or heterogeneity of the exosome population.

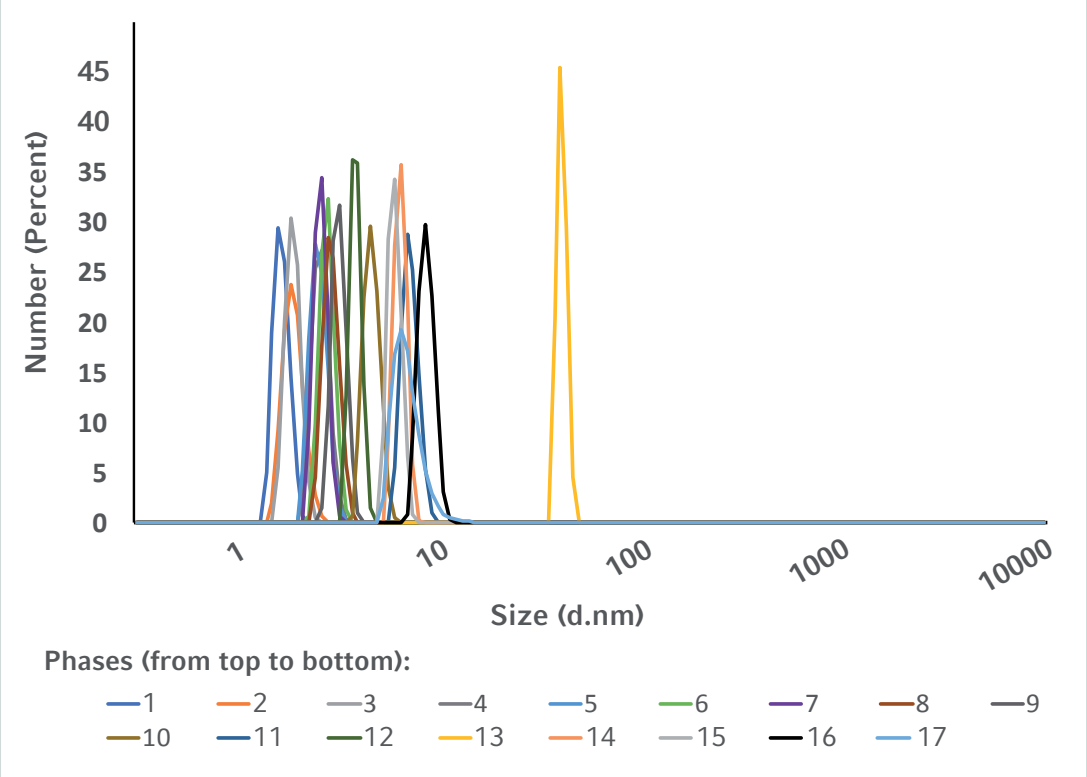

Figure 4 – Peaks detected in each fraction after centrifugation. Exosomes are detected in fraction 13.

Depending on the initial volume of media, or size of the project, several rotor solutions can be used for exosome isolation:

Rotor	Capacity	Max. speed (x *g*)
P32ST	6 x 40 mL (PET tubes)	180,000
P40ST	6 x 13 mL (PET tubes)	284,000
P56ST	6 x 4 mL (PET tubes)	409,000

Literature

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[6] Rowart P, Dufey V, Knop J, De Longueville F. Fast and Efficient Isolation of Exosomes from Stem Cells Using a Combination of Single-Use Bioreactors, High-Speed- and Ultracentrifugation.; 2023. https://www.eppendorf.com/exosomes/

Ordering information

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Description	Manufacturer	Order no.
Centrifuge CP100NX	Eppendorf	Inquire*
Rotor P32ST	Eppendorf	5720 214 003
40PET tubes	Eppendorf	5720 411 148
Rotor P40ST	Eppendorf	5720 214 002
Rotor P56ST	Eppendorf	5720 214 102

*Inquire the part number for your country

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