

OptiPrep™ density gradient centrifugation, applicable to Exosome Isolation

CP-NX Ultracentrifuge and P32ST Swinging Bucket Rotor

Exosomes are vesicles consist of a lipid bilayer membrane, their diameters are about 50 to 150 nm, and exist in body fluids such as blood, saliva, urine, amniotic fluid, and in cell culture fluids.

In recent years, it has been reported that exosomes contain various proteins and RNA (mRNA, miRNA), suggesting the potential of playing a role of intercellular communication. Utilizing this property, development as a tool of biomarker or targeted therapeutics is actively carried out.

Density gradient ultracentrifugation is effective for isolation of high purity exosomes. In this report, we introduce the OptiPrep™ density gradient centrifugation method assuming exosome purification.

Details

1. Conditions

Centrifuge : Hitachi himac Ultracentrifuge Model CP100NX

Rotor : P32ST Swinging Bucket Rotor

Centrifuge Tube: 40PET tube

Setting Speed : 24,000rpm

Maximum RCF : 100,000×g

Time : 18 hours

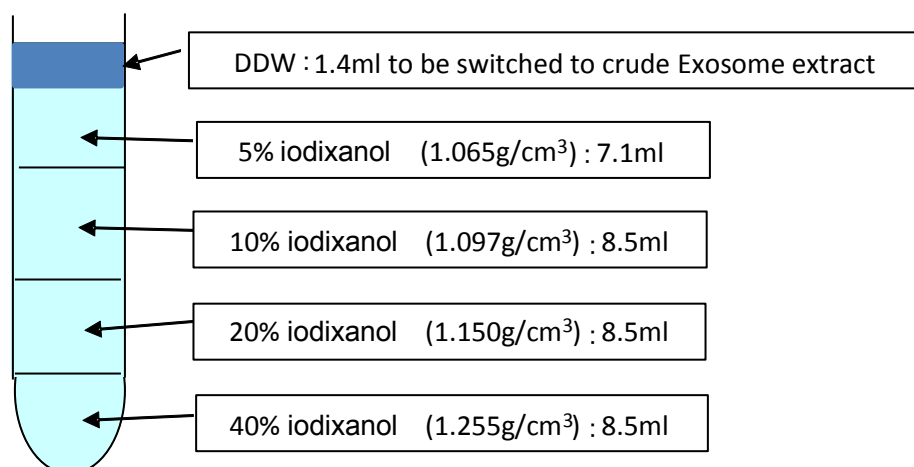
Acceleration/Deceleration : "7"/"7"

Setting Temperature : 4°C

Density gradient medium: OptiPrep™ (by AXIS-SHIELD PoC AS) *60% iodixanol in water, (1.32g/cm³)

↓
1) Dilute with 0.25M Sucrose in 30mM Tris-HCl and make 5, 10, 20, 40% iodixanol medium

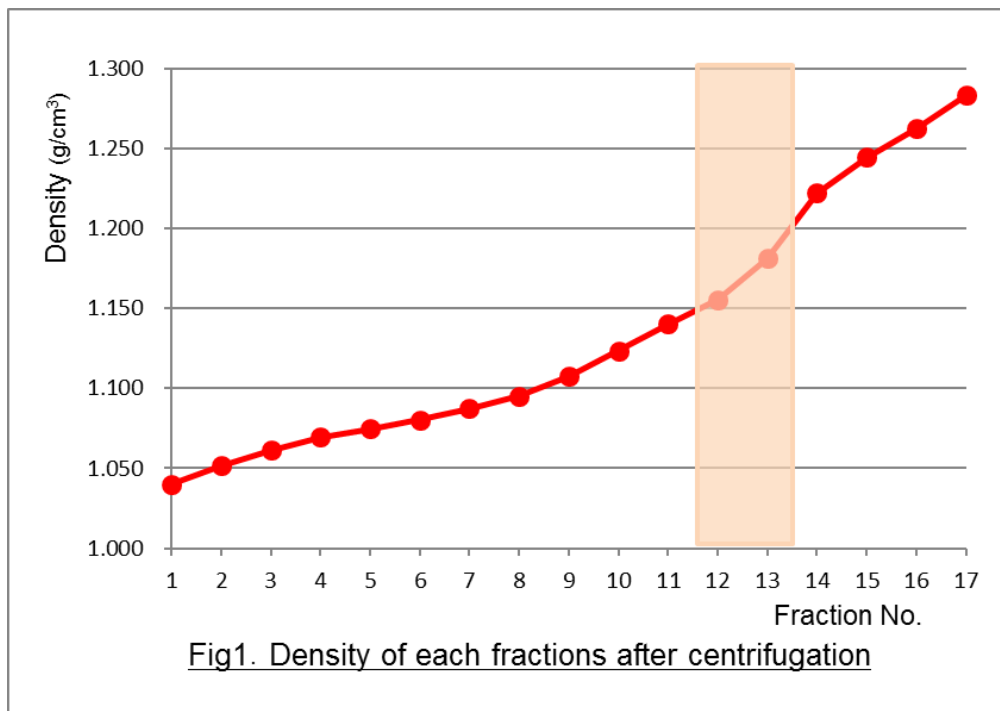
↓
2) Placing each layer into the 40PET tube as shown in the following drawing:



3) Fractionate per 2ml from the top of the tube and collect 17 fractions.

2. Results and discussion

- Figure 1 shows the results of the density gradient centrifugation, and the density gradient curve was formed.
- It is known that exosome exists in a density of 1.15 to 1.19 g / cm³ (1~3), in the fig 1, it is inferred that the exosome will be fractionated into No. 12 and No.13.



- References 1) kalra H, et al. (2013) *Proteomics*, 133, 354-3364.
2) Ji H, et al. (2013) *Proteomics*, 13:1672-1686.
3) They C, et al. (2006) *Curr Protoc Cell Biol*, Chapter 3, Unit 3.22.

Instrument



Ultracentrifuge Model CP100NX



P32ST Swinging Bucket Rotor



More Information

If you have any inquiry of this application or products, please contact us through our web site.

<http://centrifuges.hitachi-koki.com/>

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