

OptiPrep™ density gradient centrifugation, applicable to Adeno-Associated Virus Purification

CP-NX Ultracentrifuge and P32ZT Zonal Rotor

Adeno-associated virus (AAV) is a helper dependent virus, small virus of about 22 nm size without envelope. AAV is a non-pathogenic, high safety, provides long-term expression of transgenes, and applies to gene transfer method in non-dividing cell. With such features, AAV is attracting attention as a viral vector for gene therapy.

For purification of AAV, density gradient centrifugation method is used as with virus purification at the time of vaccine manufacture. In this report, we introduce OptiPrep™ density gradient centrifugation method assuming AAV purification.

Details

1. Conditions

Centrifuge: Ultracentrifuge CP100NX

Rotor: Zonal Rotor P32ZT

Setting Sped: 30,500rpm

Maximum RCF: 92,500×g

Time: 9 hours

Acceleration/Deceleration: "7"/"7"

Setting temperature: 18°C

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

- I. Dilute OptiPrep™ with DDW and make 5, 15, 25, 40, 54% iodixanol density gradient medium



- II. Put DDW first and then put each medium in order of increasing density into the rotor

DDW <small>note)</small>	100ml
5% iodixanol (1.031g/cm ³)	300ml
15% iodixanol (1.085g/cm ³)	490ml
25% iodixanol (1.137g/cm ³)	330ml
40% iodixanol (1.215g/cm ³)	235ml
54% iodixanol (1.291g/cm ³)	235ml
<hr/>	
Total	1690ml

- III. Flow rate: 30ml/min

- IV. Collection: Unloading 50ml volume, 34 fractions, feeding 60% iodixanol.

Note): In actual AAV purification, instead of distilled water, "cell lysate cultured with AAV and helper virus (adenovirus) " is used.

2. Results and discussion

- Figure 1 shows the results of the density gradient centrifugation, and the density gradient curve was formed.
- AAV's density is 1.22 to 1.26g/cm³(40~50% iodixanol).^{1)~2)} It is assumed that purified AAV will exist in light blue rows, fraction number No.28~30.

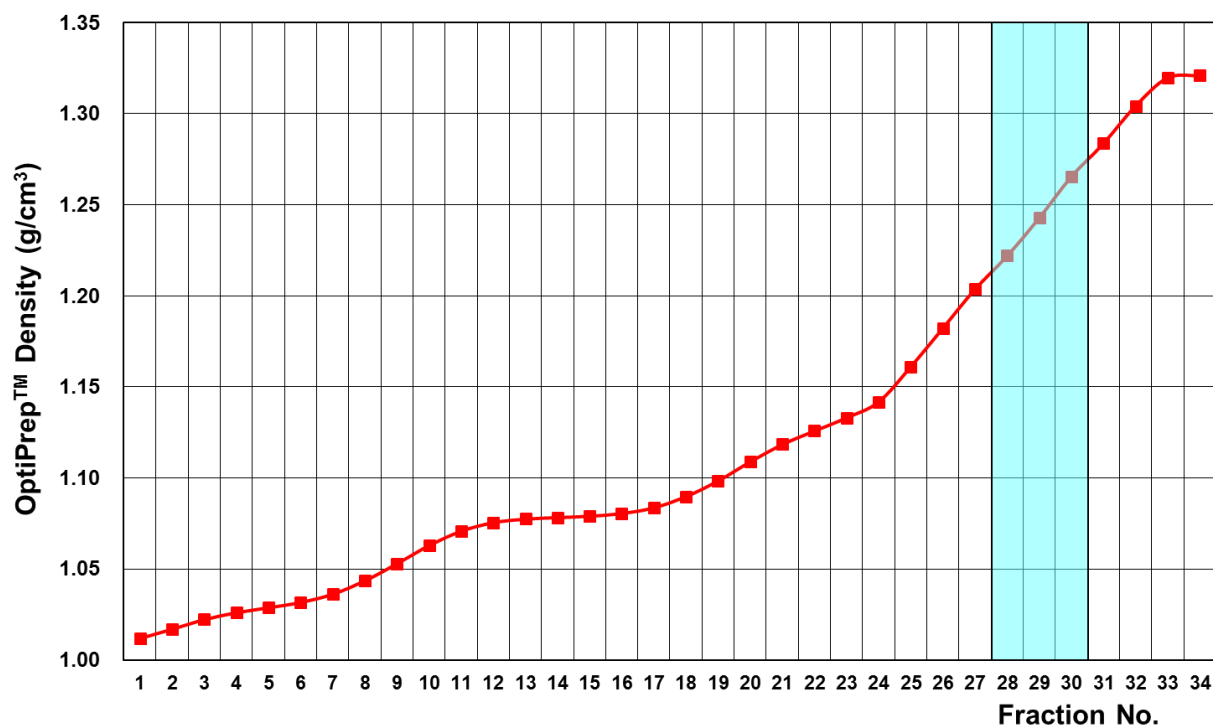


Fig.1 Density of each fractions after centrifugation

References 1): Zolotukhin S. et al. (1999) *Gene Therapy*, 6, 973-985.

2): Benjamin S. et al. (2015) *Human gene therapy methods*, Vol.26, No.4 147-157.

Instrument



Ultracentrifuge Model CP100NX



P32ZT Zonal 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/>

**Hitachi Koki Co., Ltd.
 Life-Science Instruments Division**

Shinagawa Intercity Tower A 2-15-1 Konan, Minato-ku Tokyo 108-6018 JAPAN
 Tel : (81) 3-6738-0863, Fax: (81) 3-6738-0861

*The latest information is available on our web site: <http://centrifuges.hitachi-koki.com/>