

Separation and purification of hepatitis B antigens using a zonal rotor – (3)

CP-WX series preparative ultracentrifuge/P35ZT zonal rotor

Serum proteins were removed from the small particles separated and fractionated as described in himac APPLICATION No. 131 by the sucrose density-gradient sedimentation velocity method as follows. As a result, 30 mg of HBs antigen particles whose purity was 95% or higher could be separated from 1,000 ml of human plasma.

Experiment

1. Conditions for centrifugation

Centrifuge: CP-WX series preparative ultracentrifuge

Rotor: P35ZT zonal rotor

Speed: 22,000 rpm

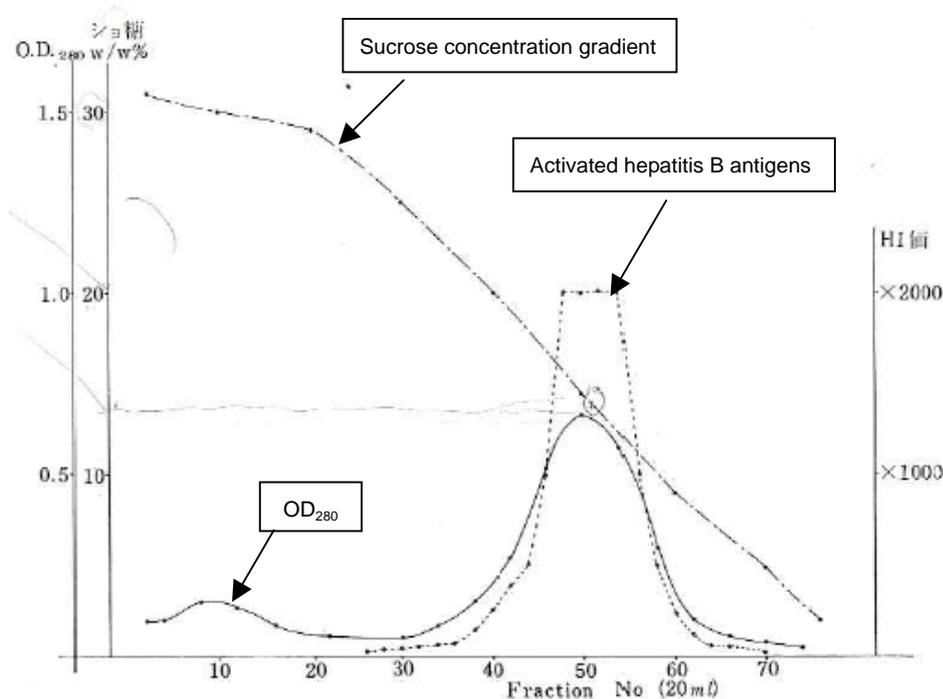
Time: 16 hours

Density gradient solution:

- (1) Pour 1,690 ml of the continuous density gradient solution whose sucrose concentration is 5 to 35% (w/w) (density: 1.018 g/ml to 1.151 g/ml) into the outer wall side of the rotor.
- (2) Add about 400 ml of solution whose sucrose concentration is 40% (w/w) to the outer wall side of the rotor to make the inner wall side of the rotor overflow certainly.
- (3) Pour 50 ml of sample into the inner wall side of the rotor. Then add about 100 ml of saline to the inner wall side of the rotor to flush out the sample remained in the pipe into the rotor completely.

Sample: Dialyze 400 ml of small particle fraction prepared as described in himac APPLICATION No. 131 to remove cesium chloride. Concentrate it to 50 ml by ultrafiltration. Add 5% (w/w) sucrose solution so that its density becomes 1.02 g/ml.

2. Result



- (1) Hepatitis B antigen particles were separated at the position of sucrose concentration 10 to 20% (w/w) (21 fractions from No. 40 to 60, 420 ml)
- (2) 30 mg of HBs antigen particles whose purity was 95% or higher could be separated from 1,000 ml of hepatitis B antigen positive human plasma by this method.

3. Explanation

While Dane particles and tubular particles having high sedimentation coefficient were sedimented, serum proteins having low sedimentation coefficient were remained in the upper layer by the density-gradient sedimentation velocity method in this experiment. Extra care must be taken when setting the centrifugation time and speed in the same manner as himac APPLICATION No. 131.

Instruments



CP-WX series preparative ultracentrifuge



P35ZT zonal rotor

For more information, visit our website at:

<http://www.hitachi-koki.com/himac.contact/index.htm>

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