HITACHI

himac APPLICATION

No. 76 June 1998

Subject Separation of total RNAs from homogenated cell line with fixed-angle rotor

Model S150AT fixed-angle rotor for preparative micro-ultracentrifuge CS150GX

Separation example of total RNAs from homogenated cell line using fixed-angle rotor only in 1.75 hours

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Most of literature describe that a separation of total RNAs from homogenated cell line has been generally processed by overnight ultracentrifugation using swinging bucket rotor.[1)] On the contrary, Hitachi has been stated that separation time for total RNAs can be reduced up to 2.5 hours by using micro-ultracentrifuge and its RP120AT fixed-angle rotor.[2)] Moreover, we have studied to reduce separation time much more by using newly developed micro-ultracentrifuge, model CS150GX of which maximum speed is 150,000rpm. As a result of the study, we could know that a speed of 150,000rpm can separate total RNAs only in 1.75 hours. By the experimental result of electrophoresis and RT-PCR, obtained total RNAs in such a rapid separation can be equivalent to that obtained by the former centrifugation.

1.Results of experiment

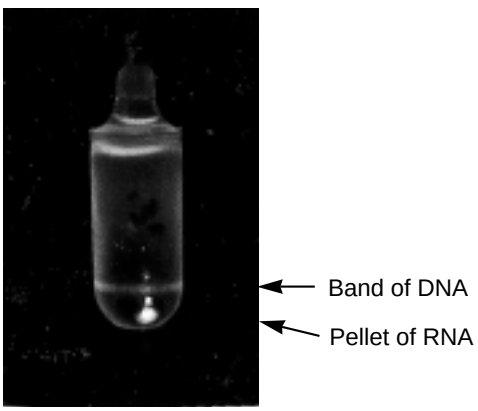
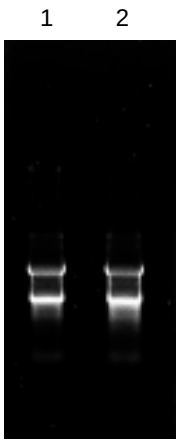
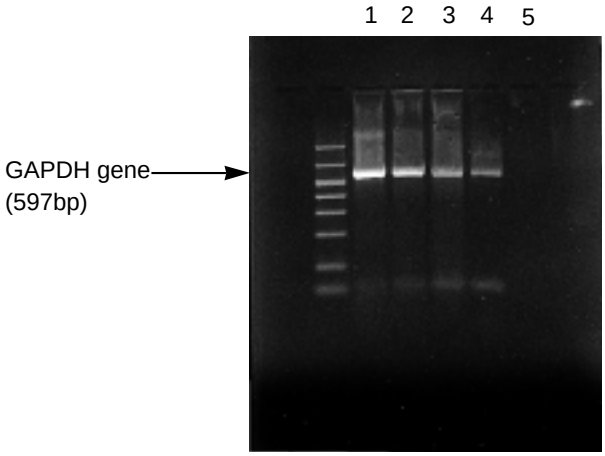


Fig.1 After centrifugation
(Finally, 1µg/ml of EtBr added)



1:Separation with S100AT6
2:Separation with S150AT

Fig.2 Result of electrophoresis



1.Molecular size marker
2.RNA separated with S100AT6 (1.5µg)
3.RNA separated with S150AT (1.2µg)
4.RNA separated with S150AT (0.7µg)
5.cDNA from leukocytes of blood (0.5µg)

Fig.3 Electrophoresis of DNA amplified by RT-PCR

2.Contents of experiment

(1) Conditions of centrifugation

Rotor	Speed	Running time	Temperature	Acc.	Dec.
S150AT fixed-angle	150,000rpm	1.75 hours	20˚C	5	7

(2) Used tube: 2PA Cone-Top tube* (* Registered trade mark of Seton Scientifie Company.)

(3) Methods of experiment

Cell pellet(5-10 x 10^6 cells/tube)
↓
4M GTC 1-1.5ml, Vortex
↓
Stack on 5.3M CsCl sol* 0.8ml
↓
Ultracentrifugation
↓
Dissolve into buffer and precipitate by ethanol

*Separation of total RNAs may not be completed due to crystallization during centrifugation when 5.7M CsCl is used.

(References)
1) Sambrook J., et al. : Molecular Cloning (2nd edition) : Cold Spring Harbor Laboratory (1989).
2) himac APPLICATION No. 40 (1993).

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