HITACHI

himac APPLICATION

No. 77 June 1998

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

Model S100NT neo-angle rotor for preparative micro-ultracentrifuge

Extraction of RNA from established cell UT-7 with S100NT neo-angle rotor and amplify and synthesize platelete factor 4 (PF4) gene by RT-PCR method.

In the current method in separation of total RNAs, overnight ultracentrifugation (approx.20hours) is generally performed with a swinging bucket rotor. On the contrary, we have been trying to shorten the separation time by raising the maximum speed of the rotors and application of fixed-angle rotors.[1), 2)] This time, we could realize speeding up the separation processing time by using a neo-angle rotor of which path-length was shorter than a fixed-angle rotor. As a result, we found that the experiment for amplification and synthesis of Platelet Factor 4: PF4[3)] could be performed by RT-PCR method using RNA separated with a neo-angle rotor as well as RNA separated with a current swinging-bucket rotor.

1.Results of experiment

(1) Sample:Established cell UT-7[4)]

(2) conditions of centrifugation

Rotor	Speed	Running time	Temperature	Acc.	Dec.
S100NT neo-angle	100,000rpm	4.0 hours	15˚C	5	7

(3) Result of electrophoresis

The result of 1.0% Agarose-Formaldehyde gel electrophoresis performed for 16 hours with 15µg of RNA is shown in Fig. 1.

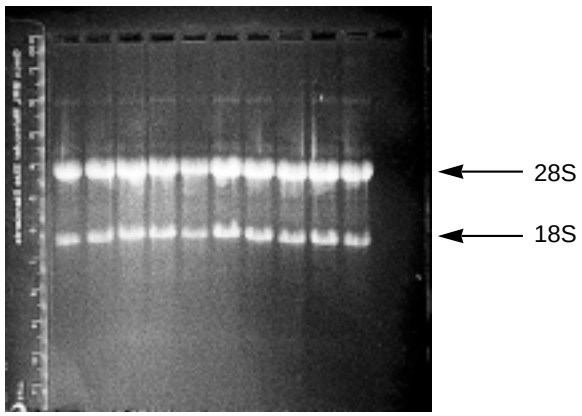

Fig.1 RNA electrophoresis

(4) Amplification of PF4 gene by RT-PCR method[5)]

Synthesis cDNA from separated 2µg of RNA with this rotor by reverse transcriptase. Moreover, synthesis PF4 gene from PCR reaction using PF4 gene primer (Fig.2).

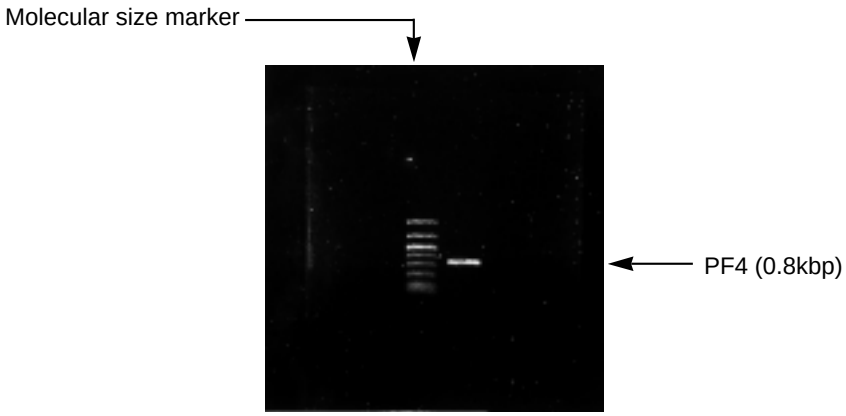

Fig.2 electrophoresis of PF4 gene

2.Methods of experiment

Cell pellet (5-10 x 10^6 cells)

↓ ← Lysate with 4M GTC sol 2.7ml

Layer the solution on 1.3ml of 5.7M CsCl (4PA seal tube)

↓

Ultracentrifugation

↓ ← Dissolve 150µl ETS buffer x 3

Presipitation by ethanol

↓

Dissolve 10-20µl DEPC'd DDW

(References)

1) HIMAC APPLICATION No. 55 (1995).
2) HIMAC APPLICATION No. 56 (1995).
3) Poncz N., et al., Blood, 69 , 219 (1987).
4) Komatsu N., et al., Cancer Res., 51 ,341 (1991).
5) Kikuchi J., Furukawa Y., et al., Blood, 89 , 3980 (1997).

HITACHI

(Export office)
Nissei Sangyo Co., Ltd.
Head Office :
1 - 24 - 14, Nishishinbashi, Minato-ku, Tokyo, 105 Japan
Tel : 81 - 3 - 3504 - 7281
Fax : 81 - 3 - 3504 - 7302

(Manufacturer)
Hitachi Koki Co., Ltd.
Scientific Instruments Div.
1060, Takeda, Hitachinaka - city, Ibaraki - pref., 312 Japan
Tel : 81 - 29 - 276 - 7384
Fax : 81 - 29 - 276 - 7475