HITACHI

himac APPLICATION

No. 61 April 1997

Subject Separation of chloroplast

Model Preparative ultracentrifuge CP α/β series

Separation of chloroplast from spinach by swinging bucket rotor P28S (holding 40ml tube)

We tried to separate chloroplast and its DNA from spinach by ultracentrifugation. The homogenate of spinach was applied to 40ml tube (about 40g of homogenate/tube) and centrifuged for 1 hour by swinging bucket rotor P28S.

Results

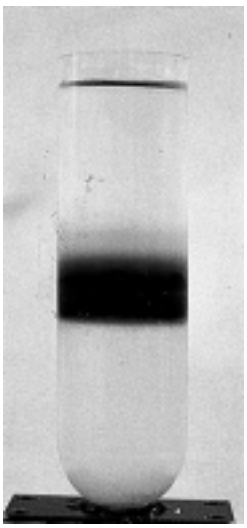

Fig. 1 : After centrifugation

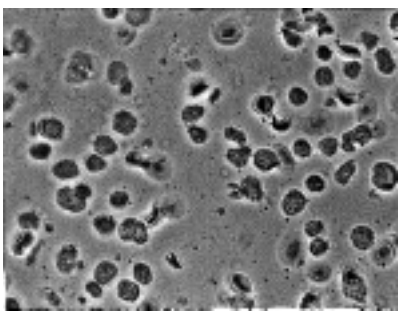

Fig. 2 : Microscope of separated chloroplast (X460)

Conditions

(1) Condition of ultracentrifugation

Rotor	Speed (rpm)	Time (h)	Temp. (˚C)	Acc. mode	Dec. mode
P28S swinging bucket rotor	25,000	1.0	4	"7"	"8"

(2) Used tube : 40PA tube

(3) Sample preparation[1)]

about 40g of spinach

↓ Add 200ml of buffer A.

homogenize

↓

filteration

↓

centrifugation

↓ CF8DL refrigerated centrifuge, 2, 500rpm, 15min, 4˚C, 50ml volume tube.

pellet (about 10g)

↓ Suspend with 6.5ml of buffer B.
Add this suspension on the sucrose solution in a 40ml tube. (See Fig. 3)

ultracentrifugation

↓ P28S swinging bucket rotor, 25,000rpm, 1h, 4˚C.

chloroplast : Fig. 1

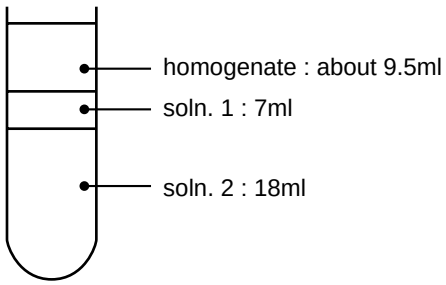

Fig. 3 Before centifugation

Buffer A : 0.35M Sorbitol, 50mM Tris-HCl (pH8.0), 5mM EDTA-Na2.
(Add 2-Mercaptoethanol and BSA to final 0.1% (v/v) just before use.)
Buffer B : 0.35M Sorbitol, 50mM Tris-HCl (pH8.0), 25mM EDTA-Na2.
Soln. 1 : 30% (w/w) Sucrose, 50mM Tris-HCl (pH8.0), 25mM EDTA-Na2.
Soln. 2 : 52% (w/w) Sucrose, 50mM Tris-HCl (pH8.0), 25mM EDTA-Na2.

Reference

1) METHODS IN ENZYMOLOGY, 118 , 167-186 (1986).

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