HITACHI

himac APPLICATION

No. 60 April 1997

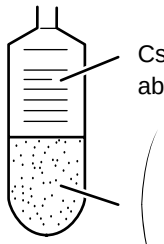
Subject Separation of plasmid DNA by neo-angle rotor

Model Preparative Micro-Ultracentrifuge CS-GX series
Neo-angle rotor S120NT

Rapid separation of plasmid DNA using 2-step gradient centrifugation by neo-angle rotor S120NT (holding 2ml tube).

We've shown in our himac APPLICATION it takes 2h to separate plasmid DNA by neo-angle rotor S120NT [1]. We tried this separation even shorter using 2-step gradient centrifugation. As a result we enabled this separation for 1 hour.

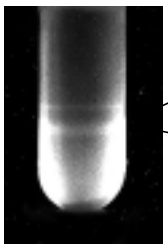
Results



CsCl : 42% (w/w)
about 0.9ml

CsCl : 0.98g
Ethydium Bromide : 40µl
sample : 0.62ml
Polyoxyethylen (10)
octylphenylether : 2µl

Before centrifugation



After centrifugation

Conditions

(1) Condition of ultracentrifugation

Rotor	Speed (rpm)	Time (h)	Temp. (˚C)	Acc. mode	Dec. mode
S120NT neo-angle rotor	120,000	1.0	20	"5"	"7"

(2) Used tube : 2PA Cone-Top tube*

*Registered trade mark of Seton Scientific Company.

(3) Sample preparation

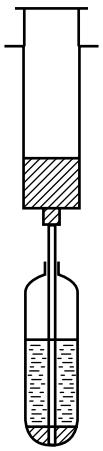
We used E.coli., JM109 holding plasmid pUC 19 DNA. The E.coli., JM109 was cultured over night and then crude DNA was isolated by alkalin-SDS method and other processes and dissolved TE buffer. We used this solution as a sample.

For each 2PA Cone-Top tube*, we provided :

(Low density solution : Upper layer)
42% (w/w) CsCl/TE buffer (pH8.0) ; about 0.9ml

(High density solution : Lower layer)
Sample: 0.62ml
CsCl : 0.98g
Ethydium Bromide (10mg/ml) : 40µl
Polyethylen (10) octylphenylether (Triton X - 100) : 2µl

Add 0.9ml of low density solution into a 2PA Cone-Top tube* using a syringe. And add about 0.9ml of high density solution gently from the tube bottom. (Use a syringe equipped to with a long needle : see below figure.) If it is not enough to fill the tube the tube should be filled with low density solution.



Reference
1) himac APPLICATION No. 59 (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