

himac APPLICATION

No. 82 June 1998

Subject Separation of plasmid DNA using micro-ultracentrifuge

Model Preparative micro-ultracentrifuge, model: CS150GX

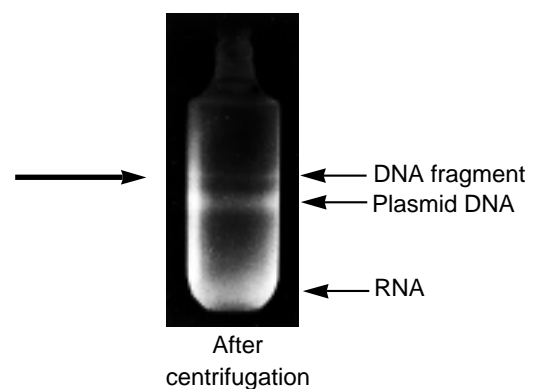
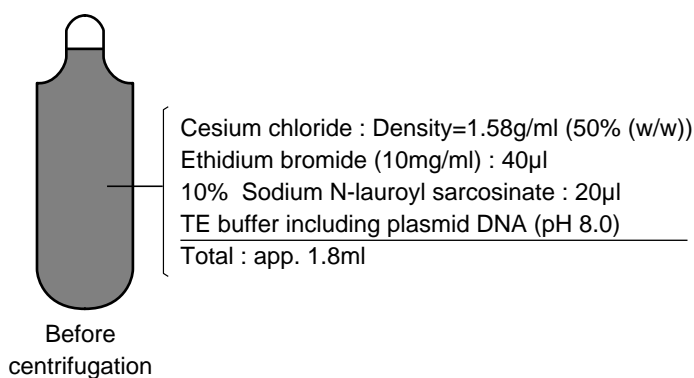
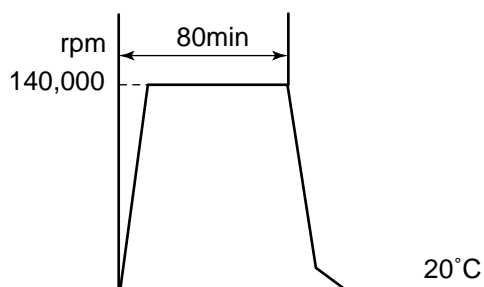
Separation of plasmid DNA with S140NT neo-angle rotor

In case of separation of plasmid DNA from E.coli with homogeneous solution of cesium chloride and ethidium bromide, ultracentrifugation for at least 2 hours with a 2ml volume tubes' neo-angle rotor used to be needed.¹⁾ However, separation time has been shortened up to 80 min. by using a newly developed neo-angle rotor of which max. speed is 140,000rpm and max.g is 752,000 x g.

When a neo-angle rotor is used for separation of plasmid DNA, RNA can be securely precipitated at the bottom of the tube by using sodium N-lauroyl sarcosinate, polyoxyethylene(10) octylphenyl ether(Triton X-100), or polyoxyethylene(23) lauryl ether(Brij 35).²⁾³⁾⁴⁾

Results of separation

- Rotor : S140NT neo-angle rotor
- Tube : 2PA Cone-Top tube *
- Run condition: Normal mode run



Separation conditions

(1) conditions of centrifugation

Roter	Speed	Running time	Temperature	Acc.	Dec.
S140NT neo-angle	140,000rpm	80min.	20°C	9	7

(2) Tube used

2PA Cone-Top tube *

(3) Sample preparation

Sample is the TE solution(10mM Tris-HCl, 1mM EDTA, pH8.0) including roughly separated plasmid DNA which was treated by alkaline-SDS method after cultivating E.coli. JM109 containing the plasmid DNA, e.g. pUC19DNA, over night.

Sample : 1.48ml
Cesium chloride : 1.52g
Ethidium bromide (10mg/ml) : 40µl
Polyoxyethylene (10) octylphenyl ether (Triton X-100) : 1-2µl
or
10% sodium N-lauroyl sarcosinate : 10 - 20µl
or
10% polyoxyethylene (23) lauryl ether (Brij 35) : 10 - 20µl

Mix above contents and insert them into a seal tube. If they can not be filled up (If there is an empty space) inside the tube, supplementary liquid(cesium chloride 1g dissolved per TE buffer 1ml) should be added to fill it up. Then, seal the tube with a tube sealer, STF-1.

* Registered trade mark of Seton Scientific Company

(References)

- 1) himac APPLICATION No.59 (1997).
- 2) himac APPLICATION No.42 (1993).
- 3) himac APPLICATION No.43 (1993).
- 4) himac APPLICATION No.45 (1995).

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