

himac APPLICATION

No. 80 June 1998

Subject Separation of plasmid DNA using micro-ultracentrifuge

Model Preparative micro-ultracentrifuge, model: CS150GX

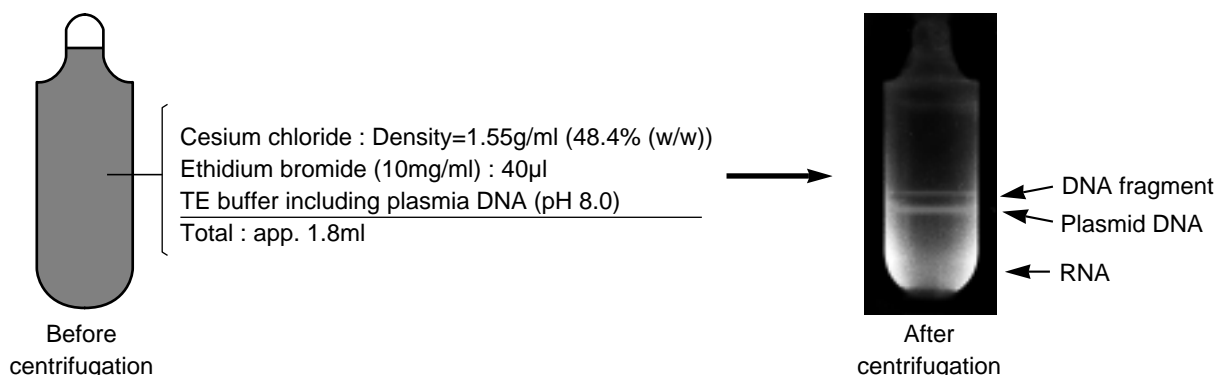
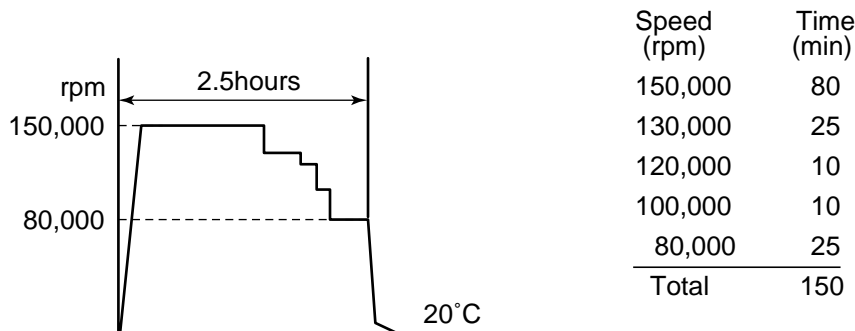
Separation of plasmid DNA with S150AT fixed-angle rotor

In case of separation of plasmid DNA from E.coli. with homogeneous solution of cesium chloride and ethidium bromide, ultracentrifugation for at least 3.5 hours with a 2ml volume tubes' fixed-angle rotor used to be needed. However, separation time has been shortened up to 2.5 hours by using a newly developed fixed-angle rotor of which max. speed is 150,000rpm and max.g is 901,000 x g.

In case a fixed-angle rotor is used, when use it with higher speed, although separation time can be reduced, users may have a problem such as crystallization of cesium chloride or unexpectedly closer distance of two bands of nicked plasmid DNA and closed circular DNA. Then, we performed multi-step operation which is programmed to decrease speed gradually to prevent cesium chloride from crystallization. We found that rapid separation of plasmid DNA could be done with high quality condition that there were no crystallization and no close of the two bands which was prevented by decreasing speed.

Results of separation

- Rotor : S150AT fixed-angle rotor
- Tube : 2PA Cone-Top tube*
- Run condition: Automatic step-mode run



Separation conditions

(1) conditions of centrifugation

Rotor	Speed	Running time	Temperature	Acc.	Dec.
S150AT fixed-angle	5-steps run	2.5 hours totally	20°C	9	7

(2) Tube used

2PA Cone-Top tube*

(3) E.coli. JM109 including plasmid pUC19 DNA

Amount of each contains per a 2PA seal tube

Sample : 1.4ml

Cesium chloride : 1.35g

Ethidium bromide: (10mg/ml) : 40µl

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

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