

# himac APPLICATION

No. 84 June 1998

**Subject** Separation of plasmid DNA with 8ml tube

**Model** Preparative micro-ultracentrifuge, model: CS-GX series

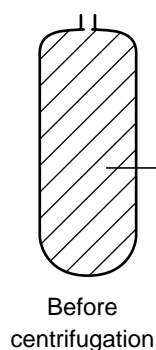
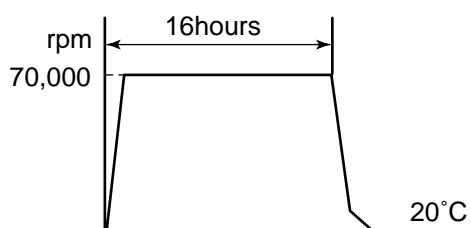
Separation of plasmid DNA with S80AT3 fixed-angle rotor

The largest rotor for the micro-ultracentrifuges up to the present can only process totally 40ml(5ml for 8). However, we newly developed S80AT3 rotor of which total processing volume is 64ml(8ml for 8). Processable volume of samples with 8PA seal tube can be equivalent to 200ml of E.coli. culture(in case separation of plasmid pUC19 DNA from E.coli. JM109. Note: below picture is showing the result of separation for the sample equivalent to 300ml of culture).

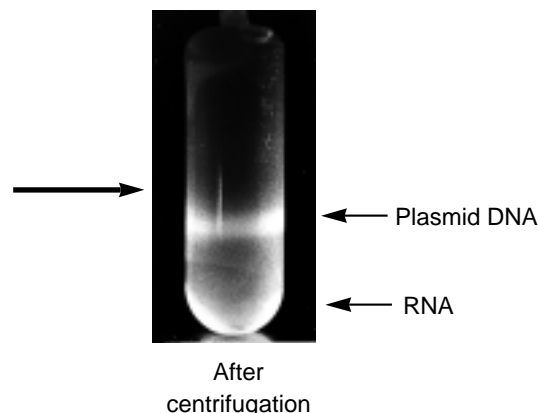
Note: Make sure not to perform overnight operation(over 5 hours) with the speed exceeding 70,000rpm, otherwise CsCl may be crystallized.

## Results of separation

- Rotor : S80AT3 fixed-angle rotor
- Tube : 8PA seal tube
- Run condition : Normal mode run



Cesium chloride : Density=1.55g/ml (48.4%(w/w))  
Ethidium bromide (10mg/ml) : 160 $\mu$ l  
TE buffer including plasmid DNA (pH8.0)  
Total : app. 8.3ml



## Separation conditions

### (1) Conditions of centrifugation

Roter	Speed	Running time	Temperature	Acc.	Dec.
S80AT3 fixed-angle	70,000rpm	16 hours	20°C	9	7

### (2) Tube used

8PA seal 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 : 5.6ml  
Cesium chloride : 5.4g  
Ethidium bromide (10mg/ml) : 160µl

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.

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