

# himac APPLICATION

No. 85 June 1998

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

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

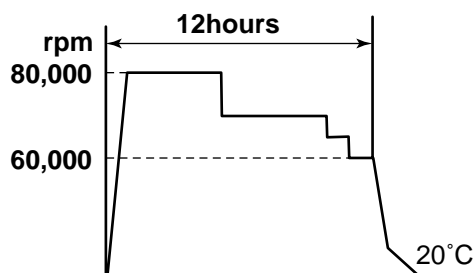
Separation of plasmid DNA by multi-step mode with S80AT3 fixed-angle rotor

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). We have established the multi-step method with this large volume rotor so that we can shorten the separation time of plasmid DNA. Moreover, because 8ml seal tube can be used with this rotor, large volume of sample equivalent to 200ml of E.coli. culture can be processed (in case separation of Plasmid pUC19 DNA from E.coli. JM109).

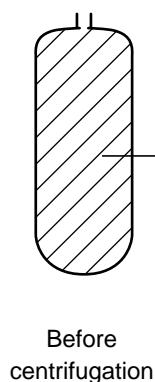
Note: below picture is showing the result of separation for the sample equivalent to 150ml of culture).

### Results of separation

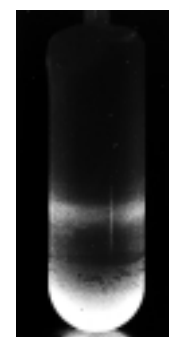
- Rotor : S80AT3 fixed-angle rotor
- Tube : 8PA seal tube
- Run condition : Multi-step mode run



| Speed (rpm)  | Time (hrs) |
|--------------|------------|
| 80,000       | 5          |
| 70,000       | 5          |
| 65,000       | 1          |
| 60,000       | 1          |
| <b>Total</b> | <b>12</b>  |



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



← Plasmid DNA  
 ← RNA

After centrifugation

## Separation conditions

### (1) Conditions of centrifugation

| Roter                 | Speed       | Running time       | Temperature | Acc. | Dec. |
|-----------------------|-------------|--------------------|-------------|------|------|
| S80AT3<br>fixed-angle | 4 steps run | 12 hours<br>totaly | 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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