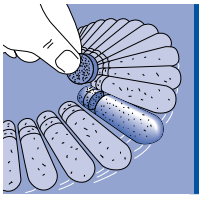

Application Information

Ultracentrifugation

A Rapid Method for Ribosome Preparation: Part 1— Using the High-Capacity Fixed Angle MLA-80 Rotor in an Optima™ MAX Tabletop Ultracentrifuge

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Introduction

The separation performance of the Optima™ MAX ultracentrifuge is demonstrated by isolating a bacterial ribosome using two high-capacity rotors. This work is described in two separate application information bulletins:

- Part 1 (this document) describes conditions for pelleting an intact 70 *S* ribosome using differential centrifugation with a fixed angle MLA-80 rotor and an 8-mL tube.
- Part 2 (document number A-1851A) describes conditions for preparing and separating 30 *S* and 50 *S* ribosomal subunits using rate zonal density gradient centrifugation with a swinging bucket MLS-50 rotor and a 5-mL tube.

Run conditions for part 1 included a final pelleting spin of 1 hr 50 min at 444,000 × *g* (80,000 rpm) and 5°C. Results show that approximately 350 mg of ribosomes can be isolated using this rotor and tube combination. A pelleting time of less than two hours allows for the processing of a ribosome preparation, from bacterial cells to final isolation, in a single day. The ribosome was characterized using analytical ultracentrifugation (Optima XL-I from Beckman Coulter) and electron microscopy.

Method

Samples of the intact 70 *S* species of ribosome were prepared from *Escherichia coli* (migula strain) following standard procedures.[1,2,3] An example of a step-wise approach used to isolate the ribosome is presented below. All procedures were performed at 5°C unless otherwise indicated.

Buffers:

A: 20 mM $MgCl_2$ 200 mM NH_4Cl, 6 mM 2-mercaptoethanol, 0.1 mM Na_2EDTA, 10 mM Tris-HCl (pH 7.4)

B: 10 mM $MgCl_2$ 500 mM NH_4Cl, 6 mM 2-mercaptoethanol, 0.1 mM Na_2EDTA, 10 mM Tris-HCl (pH 7.4)

C: 10 mM $MgCl_2$ 50 mM NH_4Cl, 6 mM 2-mercaptoethanol, 0.1 mM Na_2EDTA, 10 mM Tris-HCl (pH 7.4)

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1. *E. coli* (host for plasmid vector pBR322) were grown overnight in 2 L of LB media at 37°C with vigorous shaking (300 rpm).
2. The cells were harvested in a Avanti® J-30I high-speed centrifuge from Beckman Coulter with a JLA-8.1000 rotor using two 1 L bottles at 10,000 × *g* for 6 minutes, and the pellets pooled.
3. The bacterial pellet was resuspended in an equal volume of buffer A, then ruptured in a cell disruption bomb using nitrogen at 2,500 psi. To maximize the percentage of ruptured cells, the cells were passed through the bomb twice.
4. A solution of 1 µg/mL of DNAse in water was added to the cell homogenate.
5. The cell homogenate was divided into two aliquots and spun in two 50 mL bottles at 105,000 × *g* for 4 hours in an Avanti® J-30I centrifuge with a JA-30.50 rotor from Beckman Coulter. The supernatant was discarded.
6. The pellet from each bottle was resuspended in buffer A and split between two 8 mL tubes. A 4-mL solution of 30% sucrose in buffer B was added to each 8-mL Quick-Seal® tube, followed by careful addition of approximately 4 mL of the ribosome suspension to the layer. The material was centrifuged in an MLA-80 rotor for 1 hr 50 min at 444,000 × *g* (80,000 rpm). (Note: the tube contained a visible contaminant that banded approximately two-thirds of the way from the top of the tube.)
7. The supernatant was decanted and the clear pellet resuspended in approximately 1 mL of buffer C in a 50-mL bottle and clarified by spinning at 20,000 × *g* for 30 minutes in an Avanti J-30I centrifuge with a JA-30.50 rotor. The supernatant was dialyzed against buffer C and stored at 5°C.

The physical properties of the separation were determined with an Optima™ XL-I analytical ultracentrifuge from Beckman Coulter using a sedimentation velocity protocol. Runs were made at 30,000 rpm at 25°C in buffer C. Data were measured using interference optics and size distributions were determined using the time-derivative analysis of the sedimentation velocity profiles.[4] Further characterization of the ribosome preparation was accomplished using electron microscopy (EM). To preserve the ribosome prior to EM analysis, a sample of the pellet was treated with a solution of 2.5% gluteraldehyde (EM grade) in 0.1 M sodium phosphate buffer (pH 7.4) and stored at 5°C. The pellet was post-fixed with 2% osmium tetroxide and stained with 0.5% uranyl acetate and Reynolds lead stain. Ultrathin sections were viewed and photographed on a Zeiss model 902 microscope. Electron microscopy was performed by Dr. Megan Yao at Stanford Research Institute (SRI), Menlo Park, CA.

Results/Discussion

Analysis of the separation of bacterial ribosome showed the final pellet contained a majority of the 70 *S* ribosomes, with dissociated 30 *S* and 50 *S* subunits present only as a small percentage (Figure 1). The ribosomes provided a ratio of 2.04 for A_{260}/A_{280}. (A ratio > 1.8 for A_{260}/A_{280} is an accepted criterion for a pure ribosome preparation).[5] Electron microscopy confirmed that the isolate was highly enriched in ribosomes (Figure 2).

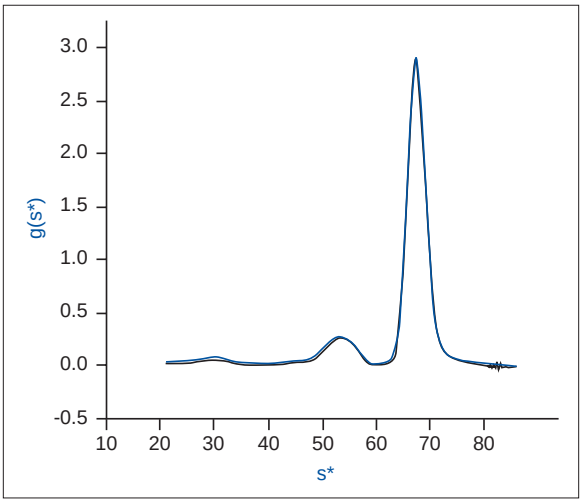

***Figure 1**. Isolation of bacterial ribosomes following centrifugation (Table 1). The size distributions are presented graphically as peaks and the preparation is shown to contain a majority of the intact 70* S *with the two subunits, 30* S *and 50* S*, present in smaller relative yields—estimated by measuring the areas under the peaks. Sedimentation coefficients (s*) were measured at 20°C and are corrected for viscosity. Error bars are included on the graph.*

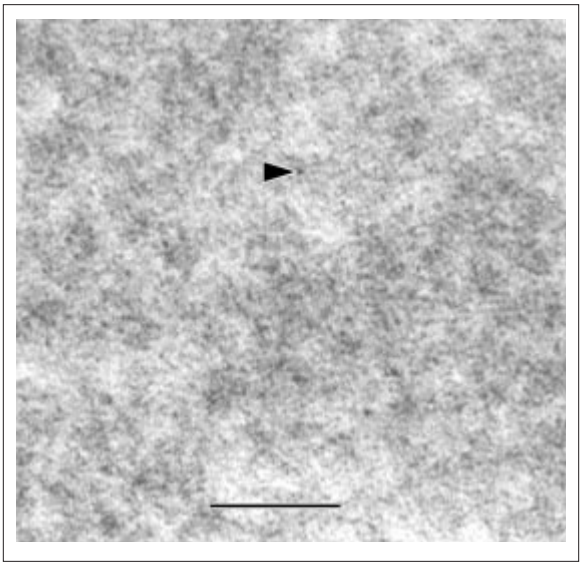

Figure 2. *Electron micrograph of 70* S *ribosomes following centrifugation (×140,000). The shape and size of the isolated ribosomes are consistent with expectations. Bar represents 0.05 µm.*

Approximately 43 mg of ribosomes were recovered from an MLA-80 rotor using a single 8-mL tube. Considering the MLA-80 has an 8-tube capacity, a maximum of 350 mg can be separated using this rotor and tube combination. Previously, the largest tube volume available for a tabletop ultracentrifuge was 5.1 mL (used with a TLA-100.4). The increased tube capacity of the MLA-80 rotor allows for a greater throughput than was possible previously. Larger tubes allow more material to be pooled and will reduce losses. Run times of less than 2 hours provide the added advantage of being able to isolate a ribosome from a bacterial mixture in a single day. Results are presented in Table 1.

Table 1. Process Capacity of a Bacterial Ribosome Using an Optima™ MAX Tabletop Ultracentrifuge

	MLA-80*
Loading	32 mL homogenate (8 × 8 mL)†
Recovery	~344 mg
Run Time	1 hr 50 min

* 444,000 × *g* at 5°C.

† In each tube, 4 mL of homogenate was layered over 4 mL of a 30% sucrose solution.

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