SV Sample Submission Guideline for Analytical Ultracentrifugation (AUC), Beckman Coulter ProteomeLab XL-I absorbance optics system

I. Sample Submission

1. Apply an account on the BCF reservation system using your official email address, which will be used to identify your affiliations. BCF will not accept service requests from public e-mail domains, such as gmail, outlook or yahoo, etc.
2. File sample submission form on-line under instrument “Analytical Ultracentrifugation - BCF”.
3. After confirming the charges and experiment time through reservation system, samples and buffers may be submitted to Miss Jin-Hsuan Yu. (Tel: 27855696 x4024, e-mail: bcf@gate.sinica.edu.tw). If BCF does not receive your samples before the scheduled time listed in the service request application form, the facility will add an extra charge for the delay, unless early notification is sent by emails more than two days in advance.
4. BCF will not compensate for your sample loss or data loss under any circumstances (hardware or software failure, operator error, or others). All experimental results are for research only. Without written permission from Academia Sinica, the user shall not claim, announce, or mislead the public into interpreting that the results of this testing is in any way related to the commercial development of the user. In addition, the user shall not in any form (including but not restricted to commercial marketing, for example advertisements, either online or offline, product packaging, catalogs, investment information etc,) use the title, logo, name, trademark or symbols that are that of Academia Sinica or similar to that of the facility, that gives the false impression of a commercial collaboration.
5. Data analysis is the responsibility of users.
6. Please refer to http://bcf.assic.sinica.edu.tw/bcf/AUC.htm for more.

II. Sample preparation

1. **Samples submitted to BCF should be non-hazardous, non-toxic and non-pathogenic. No radioactive or microbial samples are allowed.**
2. Please provide at least 450 µl for each sample. In addition, excess buffer for reference cells and for rinse is needed, at least 2 mL per different concentration.
3. Use fresh-prepared sample: samples having stored under frozen condition, gone through freeze/thaw cycles or filtered by concentration treatments (such as Centricon®) may result in aggregation, a major noise in AUC analysis! Gel filtration or extensive dialysis is recommended for buffer exchange. In order to ensure consistent results, please spin your sample at highest speed in a microfuge for 10 minutes to check for precipitation (aggregation) or to see if absorbance drops after centrifugation before submitting your samples to BCF. Please note that BCF will collect full service charges even if your sample precipitates during test run or sedimentation process, which may or may not result in insufficient OD reading.
4. Please specify the wavelength if absorbance optics is used. The absorbance at the chosen wavelength of your sample must be in the range of 0.1-1.0 OD and less than 0.2 OD for buffer alone. If experiments are abort because samples do not meet the criterion of the AUC experiment, for example, sample absorption < 0.1 or >1.0, BCF will collect service charge for that sample. Therefore, please check absorption of your samples on a reliable UV/VIS spectrometer before submission. For example, use less than 50 µM for DNA samples.
5. Avoid using DTT and 2-mercaptoethanol. If necessary, do not exceed 2 mM. Otherwise, ask for Raleigh interference detection.

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6. Please refer to “Sednterp” software to estimate partial specific volume of solute, buffer density and viscosity. Please be reminded that the viscosity and density of your buffer are needed for MW data analysis. Parameters such as partial specific volume, density and viscosity may be measured using densitometer and viscometer.
7. For details of AUC sample preparation, such as buffer selection, please refer to NIH’s experimental protocol page: https://sedfitsedphat.github.io/sedphat/experimental_protocols.htm

III. Experimental setting:

1. For samples having MW greater than 300 KDa, 40,000 rpm will be used; 30KDa to 300KDa, 50000 rpm will be used; below 30KDa, 60,000 rpm will be used. Unless specified, we will use 45000 rpm for antibodies. BCF will use the rotor speed in the sample submission form specified by users.
2. A test run at 3,000 rpm will be performed to check sample condition and cell assembly. If anything goes wrong, the cell will be reassembled.
3. Although we ensure that the cells are assembled as carefully and professionally as we can, there might be slight chance of cell leakage due to imperfection of cell components (> 16 pieces). If an accident does happen, the service charge of the sample will be waived.

IV. Stop Experiment

1. AUC experiments are stopped manually by BCF when the sedimentation is completed.
2. Please inform us in advance if the sample is to be recovered. BCF will keep your samples for one week after the experiments.
3. Raw data will be sent as an attachment which can be downloaded through the reservation system.

V. Related Softwares for AUC data analysis:

1. For links to most AUC related software, visit http://www.rasmb.org/
2. Sedfit, Sedphat could be found on https://sedfitsedphat.nibib.nih.gov/software/default.aspx
3. Ultrascan could be found on http://ultrascan.aucsolutions.com/
4. DCDT+, SVEDBERG, Sednterp could be found on http://www.jphilo.mailway.com/download.htm.
5. SEDANAL could be found on http://www.sedanal.org/.
6. US-SOMO could be found https://somo.aucsolutions.com/

HYDRO programs could be found http://leonardo.inf.um.es/macromol/programs/programs.htm

VI. Acknowledgement

Please acknowledge us if research supported and/or data generated by this instrument results in publications. For example,

“We acknowledge AUC SV data collected by Jin-Hsuan Yu in the Biophysics Core Facility funded by Academia Sinica Core Facility and Innovative Instrument Project (AS-CFII-111-201).”

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