**AAPS Interlaboratory Study**

**Tier 1 Method: Batch Dynamic Light Scattering (DLS) with Optional SLS**

## Introduction

Batch dynamic light scattering (DLS) can be used to obtain the size distribution of protein aggregates in dilute protein solutions. The primary reported results are the hydrodynamic size (diameter or radius) of the species (monomer, aggregates, particles) in the protein solutions. This method can be combined with batch static light scattering (SLS) measurements in the same instrument and the same measurement volume to determine weight-average molar mass in addition to hydrodynamic size.

Important Notes: This protocol is not a Standard Operating Protocol and assumes the required instrument is in good working order and the analysis is performed by experienced user(s). Required calibration or check standards should be run according to the manufacturer instructions. This protocol does not contain all the details of the analysis. The analyst should rely on their best judgment, routine practices, and knowledge of the technique to conduct the study; this protocol should be used as a guideline for the analysis. Samples should be analyzed immediately after thawing and preparation.

## Equipment and Materials List

- Dynamic light scattering instrument and associated consumables (please specify laser wavelengths and laser scattering angles, cuvette or plate reader, etc.)
- Protein samples and formulation buffers supplied by sample originators
    - Buffers, aliquots of unstressed, stir stressed, light stressed from Shipping #1
- Commercially available nanometer sized beads (e.g., 200 nm latex beads)
- Cleaning solutions or detergents to clean cuvettes between runs (if using cuvettes)
- 1.5 mL microcentrifuge tubes
- Pipette and pipette tips
- Vacuum and desiccator for degassing samples

## Reagents and Solutions

**Table 1:** The following samples and their buffers will be provided by sample originators. All stressed material were generated at nominally 1 mg/mL.

| Proteins | Formulations | Samples |
|---|---|---|
| Amgen IgG2 | 10 mM sodium acetate, pH 5.0 | Unstressed, 1 mg/mL<br>Stir stressed, 1 mg/mL<br>Light stressed, 1 mg/mL |
| NISTmAb IgG1 | 12.5 mM L-histidine, 12.5 mMol L-histidine HCl, pH 6.0 | Unstressed, 1 mg/mL<br>Stir stressed, 1 mg/mL<br>Light stressed, 1 mg/mL |

**Procedures**

*Instrument Setup*

1.) Follow the instrument manufacturer's guidelines to set up the instrument.
2.) Clean quartz cuvettes, flow cells, or other reusable cells as recommended. Repeat the cleaning in between loading additional samples. For disposable measurement cells, generally no preparation is required.
3.) Prepare and analyze nanometer bead standards according to the procedures generally used in your lab and manufacturer recommendations. The resulting peak(s) should have a hydrodynamic size (radius or diameter), polydispersity index, and counts consistent with past experience.
4.) Optional: If performing simultaneous SLS measurements of molar mass, calibrate the measurement cell and record solvent offsets, as recommended by the manufacturer.
5.) Report your instrument and software settings and measurement cell type (e.g., cuvette, well plate, etc.) into the **DLS Data Reporting Template Excel file.**

*Sample Preparation*

1) Thaw the samples from Shipping #1 and aliquot the Tier 1 vials (for the unstressed, stir, and light stressed mAb samples) as described in the instructions into smaller aliquots for the various methods and freeze until ready to use. Be sure to follow the procedure for vigorously mixing the contents of the vial prior to aliquoting.

2) For this analysis, thaw the aliquots designated for DLS measurements by bringing them to room temperature for no more than 15 minutes.

   Note: After thawing, particle concentration may change with time, even if the sample is maintained at 2-8˚C. This change may be mitigated by analyzing the samples immediately (within 2 hours) of thawing.

3) A proper mixing of the samples prior to analyzing is important to eliminate particle clumping. Prior to dilution of each sample, mix each aliquot (mentioned in #2) by pipetting the sample with a 1000 uL tip near the bottom of the sample tube 10 times in different directions but not touching the bottom or creating bubbles. Remove sample from near the bottom of the vial, from about ¾ of the depth of the tube from the top of the liquid before dispensing the sample into another container containing the buffer.

4) Some samples might appear turbid and may need to be centrifuged or briefly spinned down to remove the large particles that might interfere with the measurement. Perform this step as you would routinely in your lab.

5) Load each sample into a clean measurement cell and cap the cuvette to prevent dust contamination and solvent evaporation.

*Sample Measurement*

1.) Load the sample into the measurement cell so as not to introduce air bubbles. Load the cell into the instrument and allow the sample to equilibrate to the instrument temperature for about 5 minutes.

2.) Perform a measurement; multiple measurements may be taken for each "load". For all measurements, use the manufacturer's recommended settings and/or organizational best practices for attenuation settings, acquisition time, number of acquisitions, etc., as applicable. The specific measurement parameters (as shown in Table 1) should be recorded in the **DLS Data** reporting Excel file.

    - Check that the mean scattering intensity (count rate) reported during the measurement is according to the manufacturer's recommendation.
    - Check the autocorrelation function to learn about the quality of DLS measurements.
    - Employ data filtering according to normal best practices.
    - If one measurement appears to be an outlier or is affected by dust, bubbles, etc., it may be discarded and an additional measurement performed.
    - Optional: Record static light scattering intensity and weight-average molar mass by static light scattering simultaneously with DLS data.

3.) Repeat step 1 and 2 for a total of 3 replicates (9 measurements).

4.) Repeat steps 1-3 for all samples.

Table 1. Important instrument and measurement parameters

<table>
<tr><th>Parameter</th><th>Values</th></tr>
<tr><td>DLS detector angle</td><td>Per manufacturer</td></tr>
<tr><td>SLS detector angle (optional)</td><td>Per manufacturer</td></tr>
<tr><td>Acquisition time</td><td rowspan="2">20 s or per manufacturer's recommendation or organizational best practices</td></tr>
<tr><td>Number of acquisitions</td></tr>
<tr><td>Total time for a single measurement</td><td>As specified above or (acquisition time)×(number of acquisitions), typically 20-100</td></tr>
</table>

| | |
|---|---|
| | s |
| Equilibration time before measurements begin | 60 s |
| Delay between measurements | 0 s |
| Volume inside cuvette | According to manufacturer's instructions |
| Positioning Method | Seek for optimum position if applicable or N/A |
| Automatic Attenuation selection | Yes |
| Temperature | 25 ˚C |
| Refractive index of dispersion (solvent) | 1.33 |
| Refractive index of sample | 1.41 (for protein) |
| Viscosity of medium (at 25 °C) | 0.89 cP |
| dn/dc (mL/g)<br>(optional, for SLS only) | 0.187 |

**Understanding Results**

Data and selected instrument parameters used should be entered in the **DLS Data** reporting template. For each sample, report the mean counts, mean Z-average radius or diameter, and mean polydispersity index along with standard deviations. Report the intensity based mean hydrodynamic radius or diameter for each peak along with standard deviations.

For each sample, check the raw correlation data to ensure that the amplitude is stable and correlograms decay to a flat baseline. Fluctuating amplitudes or noisy correlograms indicate foreign particles or dust, aggregation of the sample, poor thermal convection, solvent evaporation, or dirty cuvettes. If sediment is visible at the bottom of a cuvette following measurement, then that data should be discarded. If there are anomalies in any scan or acquisition, the analysts may use their best judgment to decide whether that scan should be eliminated or not.

## Further Information

For any specific questions regarding this method, please reach out to Sophia Kenrick at skenrick@wyatt.com. Please copy aapsinterlab@nist.gov on your email.