**Signature Page**

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| --- | --- | --- | --- | --- |
| **Title** | Characterization of AAV Aggregation by Dynamic Light Scattering | | | **FDBT** |
| **Department** | VAD | **Protocol Number** | **FF031-A-01-AD-01** |

To be completed ***prior*** to performing the run:

|  |  |  |  |
| --- | --- | --- | --- |
| **Role** | **Name, Title** | **Signature** | **Date** |
| **Author** | Nathaniel Dziuba, Sci II |  |  |
| **Approver** |  |  |  |

**Approver**: By approving this document, I agree to the appropriateness of this protocol

To be completed ***after*** performing the run and data analysis:

|  |  |  |  |
| --- | --- | --- | --- |
| **Role** | **Name, Title** | **Signature** | **Date** |
| **Analyst** |  |  |  |
| **Reviewer** |  |  |  |

**Reviewer**: By signing this document, I acknowledge that I have reviewed the executed protocol, data, and results

# PURPOSE

Dynamic light scattering will be used to determine the extent of AAV aggregation.

# BACKGROUND

Samples will be generated from the Fujifilm PD team for analysis. Samples will be plated at varying dilutions and be analyzed with a Wyatt Dyna Pro Plate Reader III. Standard calibrants will be used to determine the distribution range of the aggregates. The expected size of the molecule of interest is approximately 16-20 nm in diameter.

# VIRTUAL KIT REFERENCE

|  |  |
| --- | --- |
| **Description** | **Virtual Kit Number** |
|  |  |

# EXPERIMENTAL METHODS

* 1. Variables

| **Name** | **Notes** |
| --- | --- |
| Sample dilutions | Multiple sample dilutions will be tested to determine optimal testing dilution |

* 1. Set-points/Target Parameters

| **Name** | **Notes** |
| --- | --- |
| Plate Reader | 25℃ |

* 1. Controls

| **Name** | **Notes** |
| --- | --- |
| Control Beads | Beads purchased that have been manufactured at select sizes and distributions. Will be used as a positive control for detection. |
| RM | Reference Material will be used as a process control. |
| Diluent | The diluent used for the serial dilution. |

* 1. Sampling Plan

|  |  |  |
| --- | --- | --- |
| **Sample Name** | **ID/Description** | **Lot Number** |
| Ctrl1 | Unfiltered Diluent: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  |
| Ctrl2 | 0.02-micron filtered Diluent: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  |
| Bead1 |  |  |
| Bead2 |  |  |
|  |  |  |
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# DEFINITIONS

# SAMPLE TERM - Definition not bold, ideally in alphabetical order. Must list out for all abbreviations used in the document

* 1. **CV**: Coefficient of Variance
  2. **AAV**: Adeno-Associated Virus
  3. **DLS**: Dynamic Light Scattering
  4. **%PD**: Polydispersity Index
  5. **ACF**: Auto-correlation function
  6. **PBS**: Phosphate Buffer Solution

# INSTRUMENTATION AND EQUIPMENT REQUIRED

* 1. Required instruments and equipment table:

| **Equipment** | | **Asset ID #** | **Calibration Due Date** |
| --- | --- | --- | --- |
| BSC | |  |  |
| Integra Assist Plus | |  |  |
| DynaPro Plate Reader III | |  |  |
| Plate Centrifuge | |  |  |
| Multi-channel Pipette | |  |  |
| Micropipettes | P: |  |  |
| P: |  |  |
| P: |  |  |
| P: |  |  |

# COMPONENTS REQUIRED

* 1. Reagents
     1. PBS: Gibco, Cat # 10010-023
  2. Materials
     1. Plates: Corning 384 well plate low volume, non-treated and black w/ clear bottom, Cat # 3540, or equivalent
     2. Filters: GE 0.02-micron filter, Cat # 6809-2002, or equivalent
     3. Standard Beads:
        1. 20 nm Beads, ThermoFisher Cat# 3040A, or equivalent
        2. 125 nm Beads, ThermoFisher Cat# 3125A, or equivalent
     4. Syringes; any sterile luer-lock tipped syringe
     5. Plate Sealing Tape; ThermoFisher Cat# PI15036
     6. Centrifugal Filters:

| **Reagent/Material** | **Supplier/**  **Manufacturer** | **Catalogue Number** | **Lot Number** | **Expiration Date** |
| --- | --- | --- | --- | --- |
| Diluent: |  |  |  |  |
| Control Bead: |  |  |  |  |
| Filters |  |  |  |  |
| Syringe |  |  |  |  |

# REFERENCES

# Reference Documents:

| Number | Title |
| --- | --- |
| FDBT-SOP-0372 | Good Documentation Practice (GDP) |
| FDBT-SOP-0388 | Deviation Management |
| FDBT-SOP-0401 | Conducting Out-of-Specification (OOS) Investigations in the Quality Control (QC) Laboratories |
| FDBT-SOP-0413 | Performing Statistical Outlier Tests for Quality Control Assays |
| FDBT-SOP-0429 | Reagent and Solution Preparation for the Quality Control Laboratory |
| FDBT-SOP-0431 | Handling of Invalid Test Results, Invalid Assays and Aborted Assays in the Quality Control Laboratories |
| FDBT-SOP-0434 | Gowning Requirements for the Quality Control Laboratories and Cleaning of PCR Labs at TBF B100 |
| FDBT-SOP-0441 | Guidelines for Four-Phase On-the-Job Training of Analytical Analysts in Quality Control |
|  |  |

# PROCESS FLOW DIAGRAM

|  |
| --- |
|  |

# SAFETY CONSIDERATIONS

* 1. Waste disposal
     1. List disposal guidelines for any reagents, materials, and samples in the protocol

# PROCEDURE

* 1. General Notes:
     1. Determine what the formulation buffer for the sample is.
        1. If the buffer can be appropriately modeled in SEDNTERP use the filtered formulation buffer for dilutions. OR if the formulation buffer is similar in composition to another solution, use the formulation buffer for dilutions and use parameters from the other solution for modeling.
        2. If the formulation buffer cannot be modeled in SEDNTERP use PBS or a formulation buffer composition that can be modeled. (i.e., if detergents cannot be modeled, but all other components can be, remove the detergent). **Use this composition and perform a buffer exchange with spin columns.**

**Note: Small amounts of detergent (e.g., 100 ppm) should not pose a problem when calculating the viscosity due to the very small amount added. In this case, use the formulation buffer composition without the detergent or offending analyte when computing the viscosity.**

* + 1. Obtain a protein concentration for the samples to be tested. This value will be used to help determine the optimal dilution range to start with is 10 - 1 mg/mL.
       1. Future runs may use capsid or genome copies instead if using purified product.
       2. In process samples (i.e., “dirty” samples) may require a series of dilutions to test.
    2. If the protein concentration is unknown perform a full 2-fold series dilution from neat, 2-128-fold dilution.
    3. For each unique diluent used a filtered and unfiltered control must be added to the sample plate.

**Note: This step must be followed.**

* 1. Diluent Preparation:
     1. Filter the selected diluent through a 0.02-micron filter inside a BSC.
  2. Sample and Controls Preparation:
     1. Sample Preparation: Diafiltration
        1. If the formulation buffer cannot be modeled perform a buffer exchange into a buffer that can be modeled.
        2. Add 150 uL of sample to a 10kDa regenerated cellulose spin column (Amicon) and spin at 14,000g for 10 min,
        3. Add 150 uL of dilutant to the top of the filter and spin again for 10 min.
        4. Perform the dilutant addition for a total of 3 times.
        5. Recover the filter retentate and dilute to a final volume equal to 150 uL.
        6. Follow the manufacture’s protocol for instruction on how to use this filter membrane.
     2. Pre-Test: System Suitability
        1. Before samples are run a simple system suitability test can be run on the system
        2. While inside a BSC use Table 1. for the dilution scheme and prepare the control beads within a plate for a system suitability check.
        3. Seal the plate
        4. Run the plate on the instrument or store at room temperature and use within 24-hours.
        5. System Suitability – Pass:
           1. Proceed to the next step.
        6. System Suitability – Fail:
           1. Reboot the instrument and retest the plate.
           2. If above also fails, remake the plate.
           3. Contact the vendor if no user resolution if found.
        7. **Note: NIST beads will be used to perform the system suitability. System suitability wells in the plate can be filled 24-hours prior to performing the system check.**
     3. Sample and Controls Preparation:
        1. Use the same dilutant to dilute each sample
        2. Dilute the sample and control beads according to Table 1.
        3. The sample and plate preparation can be performed on the Integra Assist Platform.

**Table 1. Control and Sample Dilution Table**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample concentration | Final Concentration | Sample volume µL | Dilutant µL | Total Volume µL |
| Sample/Control Beads/Buffer Controls/RM | Neat | 100 | 0 | 100 |
| 1:2 | 100 (Neat) | 100 | 200 |
| 1:4 | 100 (1:2) | 100 | 200 |
| 1:8 | 100 (1:4) | 100 | 200 |
| 1:16 | 100 (1:8) | 100 | 200 |
| 1:32 | 100 (1:16) | 100 | 200 |
| 1:64 | 100 (1:32) | 100 | 200 |
|  | 1:128 | 100 (1:64) | 100 | 200 |

* 1. Plate Preparation:

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample Name |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 25nm Beads | A | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| 125nm Beads | B | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| RM | C | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| Sample #1 | D | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| Sample #2 | E | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| Unfiltered Buffer | F | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| Filtered Buffer | G | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| Sample #3 | H | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |

* + 1. Prepare a 384 well plate according to the example design below with 25 uL of material in the well:
    2. The system suitability plate can be used for the sample plate, or a new plate can be used if needed.
       1. If a new plate is used, be sure to add the control beads, always run control beads in every plate with sample
    3. Cover the top of the plate with an adhesive seal.
    4. Briefly centrifuge the plate for 800 rpm for 3 minutes to remove air bubbles. Precaution should be taken to not touch the bottom of the plate.
    5. Place the plate into the plate reader and set up the instrument for analysis
  1. Software Setup:
     1. Reboot the software if the software is open upon walk-up.
        1. Not rebooting the software can cause issues in instrument communication, measurement acquisition, and other issues.
  2. Sample and Solvent Selection:
     1. In the software select the Sample tab and select an appropriate sample type and select the assign button
     2. For the solvent select a representative composition towards the matrix. As stated in the general notes, if the viscosity and density cannot be modeled with SEDNTRP or is not like another solution, perform a diafiltration
  3. Analysis Parameters: Setup the instrument parameters according to the table below

|  |  |
| --- | --- |
| Fixed Parameters | |
| Calculate D10/D50/D90 | Yes |
| Instrument | |
| DLS Acq Time | 10 |
| DLS Acq Number | 10 |
| Set plate to | 384 |
| Plate Sealant | Sealing Tape |
| Auto-Attenuation | Yes |
| Plates | |
| Select plate type | Corning 3540 |
| Experimental Designer | |
| Fixed Temperature | 25℃ |
| Cell Measure Number | 1 time |
| Auto-Attenuation | Enable |
| Well Image | Enable per well |
| Acquisition Time | 10 seconds |
| Acquisition Number | 10 |
| Label Measurements By | Well Name |
| Sealing Tape | Yes |
| Leave Laser On? | Off (Disable) |
| Summary | |
| Experiment Type | Isothermal |
| Auto-Attenuation | Enabled (Yes) |
| Image Each Well | Enabled (Yes) |
| DLS acquisition Time | 10 seconds |
| DLS acquisition per measurement | 10 |
| Measure SLS | NO |
| Measurements per well | 1 |
| Number of scans | 1 |
| Set Temperature | 25℃ |
| Plate Sealant | Sealant Tape |
| End of Experiment Laser On | Disable (No) |

* 1. Data Filtering
     1. Use the criteria for data filtering listed in the table below. This information is listed as an example and can be modified by the scientist as needed:

|  |  |
| --- | --- |
| Data Filtering | |
| Unselect Intensity Fluctuation | N/A |
| Select Minimum Amplitude | 0.05 |
| Unselect Maximum Amplitude | N/A |
| Unselect Amplitude Fluctuation | N/A |
| Select Baseline Limit (1 +/-) | 0.01 |
| Select Maximum SOS | 10 |
| Select Minimum Rh (nm) | 0.5 |
| Unselect Maximum Rh (nm) | N/A |

* 1. Outlier Calculations:
     1. Perform an outlier test per FDBT-SOP-0413 if one of the triplicate wells is suspected of being an outlier. ­­

# TECHNICAL AND OPERATIONAL OBSERVATIONS

* 1. General Assessment of Data Quality
     1. View each image of each well taken and look for the observation of bubbles
     2. The intensity (amplitude) of the auto-correlation should be > 1.0
     3. The auto-correlation function decay should return to baseline (1.0)
        1. If baseline is not achieved reanalyze the sample by reassigning the acquisition time and number of acquisitions

Assay Acceptance Criteria:

* + 1. SST: (20 nm bead standard): Average diameter of 20 nm +/-0.5 from a triplicate reading
       1. %RDS < 5%
    2. Assay Acceptance: RM has an average hydrodynamic radius of 13.6 +/- %5.0 nm
       1. RM has a %RSD < 5%
       2. RM has a %PD < 15%

Record the file name of the raw data and the file path of where the data is to be stored in the section below:

|  |  |
| --- | --- |
| File Name |  |
| File Path |  |

# NOTES

|  |
| --- |
| Capture observations, modifications/corrections to the protocol/procedure, troubleshooting actions, etc. |

1. **ATTATCHMENTS**

|  |  |
| --- | --- |
| **Attachment** | **Description** |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |