PROTOCOL

AAV Quantification Using Gator® High Sensitivity AAV Kit Part No. 350003

Scope

This document provides a detailed protocol for running a quantification assay for adeno-associated virus (AAV) particles using Gator® High Sensitivity AAV Kit and analyzing AAV quantification data using the GatorOne software. It also includes common issues and troubleshooting tips.

Introduction

Adeno-associated viruses (AAV) are non-enveloped viruses with a small single-stranded DNA genome. AAV is widely used in viral gene therapy since it is non-integrating and non-immunogenic, reducing the risk for insertional mutagenesis in the host genome or an immune response. Gator® High Sensitivity AAV Kit along with Gator® Biolayer Interferometry (BLI) technology is highly useful for the quantification of low titer samples of AAV viral particles (vp) from different AAV serotypes. Quantification through BLI offers many advantages over an ELISA such as a simpler assay format, reduced assay run time, and decreased hands-on labor, thus minimizing user-dependent variability. The kit has a limit of quantitation (LoQ) of 5E+06 viral particles (vp)/mL and an limit of detection (LoD) of 1E+06 vp/mL. The dynamic range is over four orders of magnitude (5E+06 – 5E+10). The kit enables the quantification of 8 samples in 35 minutes.

Materials Required

- Gator® High Sensitivity AAV Kit, Part No: 350003
- Gator® Max Plate, Part No: 130062
- Gator® BLI 96-Flat Plate, Polypropylene, Part No: 130260 (Case)
- Precision pipettes and tips
- Tweezer, Fisher Scientific, Cat No: 14955032

Storage

Store HS AAV probes at room temperature (RT) in the foil packaging pouch, ensuring that the zipper is fully sealed to avoid humidity and moisture contamination. Probes are stable at RT for 1 year. Store the HS AAV Reagent Set at 4°C; the unopened reagent set is stable up to 6 months. Once the reagents are diluted, the kit is stable at 4°C for 1 month.

HS AAV Kit Quantification Protocol

Reagent Preperation

1. Bring the HS AAV Solutions A, B, and C and the Quantitaton (Q) Buffer to RT.
2. When using the kit for the first time, prepare the working solution of reagent A and B by diluting each of them in 18 mL of Q buffer. Mix thoroughly.

PROTOCOL

AAV Quantification Using Gator® High Sensitivity AAV Kit Part No. 350003

Max Plate Setup

Note: Each fresh Max Plate can accommodate 24 probes over 3 consecutive assays.

1. Pipette 250 uL/well of Q Buffer into columns 1-10 of a new Max Plate. If setting up 2 or 3 consecutive assays, add Q buffer into columns 11 and 12, respectively.
2. Using a probe picker, pick out fresh probes from the tray and place them into column 10. For assays 2 and 3, repeat for columns 11 and 12.

Max Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A										Q Buffer + Probes (For Assay 1)	Optional: Q Buffer + Probes (For Assay 2)	Optional: Q Buffer + Probes (For Assay 3)
B												
C					Q							
D					Buffer							
E												
F												
G												
H												

AAV Standard Preparation

Prepare a standard curve for a particular serotype when using the kit for the first time. Standard curves for each serotype can be saved for the future.

Refer to table below to determine the optimal AAV standard range. The recommended range for each serotype is:

AAV1	2E+07 vp/mL – 1E+11 vp/mL
AAV2	5E+07 vp/mL – 1E+11 vp/mL
AAV3	4E+06 vp/mL – 1E+11 vp/mL
AAV4	8E+07 vp/mL – 1E+11 vp/mL
AAV5	1E+06 vp/mL – 5E+10 vp/mL
AAV6	2E+06 vp/mL – 1E+11 vp/mL
AAV8	5E+07 vp/mL – 1E+11 vp/mL

AAV Quantification Using Gator® High Sensitivity AAV Kit Part No. 350003

Quantification Plate Setup

Vortex all of the reagents prior to pipetting them EXCEPT for Solution C. Prepare the Black Plate according to the following plate design.

1. Add 180 uL/well of standards/samples into column 1 of a new Black Plate.
2. Add 180 uL/well of HS AAV Solution A into column 2 of the Black Plate.
3. Add 180 uL/well of HS AAV Solution B into column 3 of the Black Plate.
4. Add 180 uL/well of HS AAV Solution C into column 4 of the Black Plate.
5. For assays 2 and 3, repeat steps 1-4. For columns 5-8 and 9-12, respectively, use samples instead of standards.

Note: Each new Black Plate can accommodate 24 samples for 3 consecutive assays.

Black Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A					(Optional Assay 2)				(Optional Assay 3)			
B												
C	Sample /	Solution	Solution	Solution								
D	Standards	A	B	C								
E	180 uL	180 uL	180 uL	180 uL								
F	Assay 1											
G												
H												

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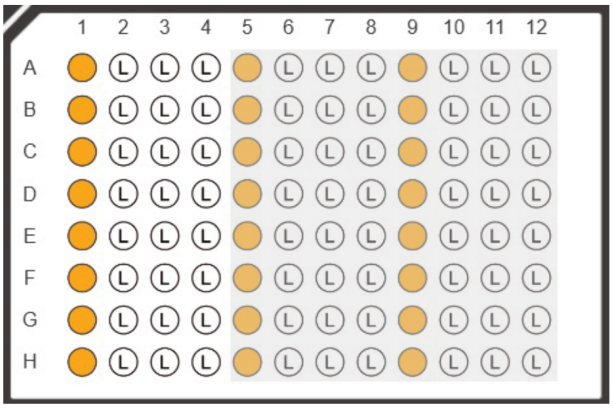
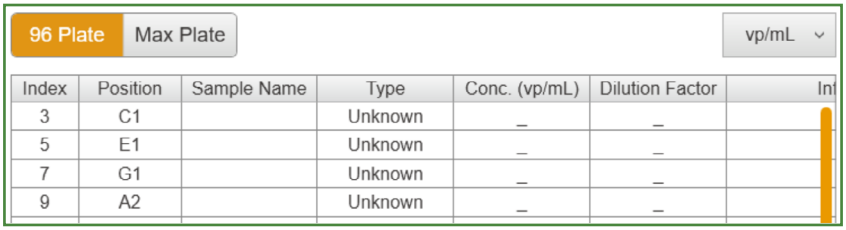
AAV Quantification Using Gator® High Sensitivity AAV Kit Part No. 350003

Assay Setup on Gator® Prime or Gator® Plus Instrument

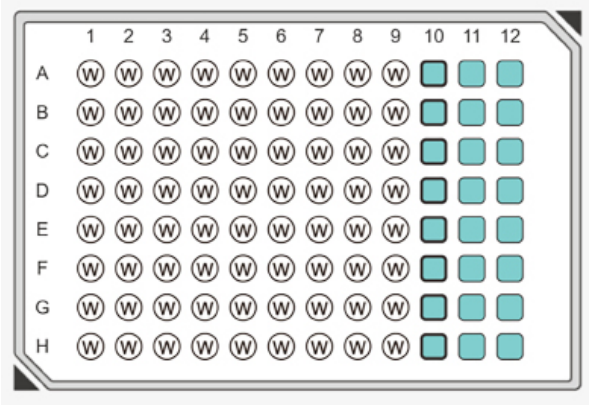
1. Open the lid of the instrument.
2. Place the Black Plate into Shaker A and the Max plate into Shaker B. Column numbers should be oriented to face the right side.
3. Close the lid.
4. On the Quick Start menu in the GatorOne software, select “K assay” to set up a new experiment and name your assay.
5. Under the Description tab, input assay details and description.
6. Under the Basic Parameters tab, specify the following parameters:
   - Data Acquisition: 5 Hz
   - Shaker Setting: Flat – A & B at 30°C
   - Pre-wet and Pre-Mix Setting: 180 sec
   - Shaker A at 0 RPM and Shaker B at 1000 RPM
7. Under Plate Set Up, set up the 96-well plate map. Refer to image below.
   - Highlight columns 1 on the 96-well plate and select the Sample icon.
   - Highlight columns 2, 3, and 4 on the 96-well plate and select the Load icon.



   - Assign each well its position with the name of the corresponding sample/solution. Refer to the image below.
   - Assign the known concentrations for the standards under the 96 Plate description box.
     Note: Repeat step 7 for columns 5-8 and columns 9-12 for each consecutive assay.



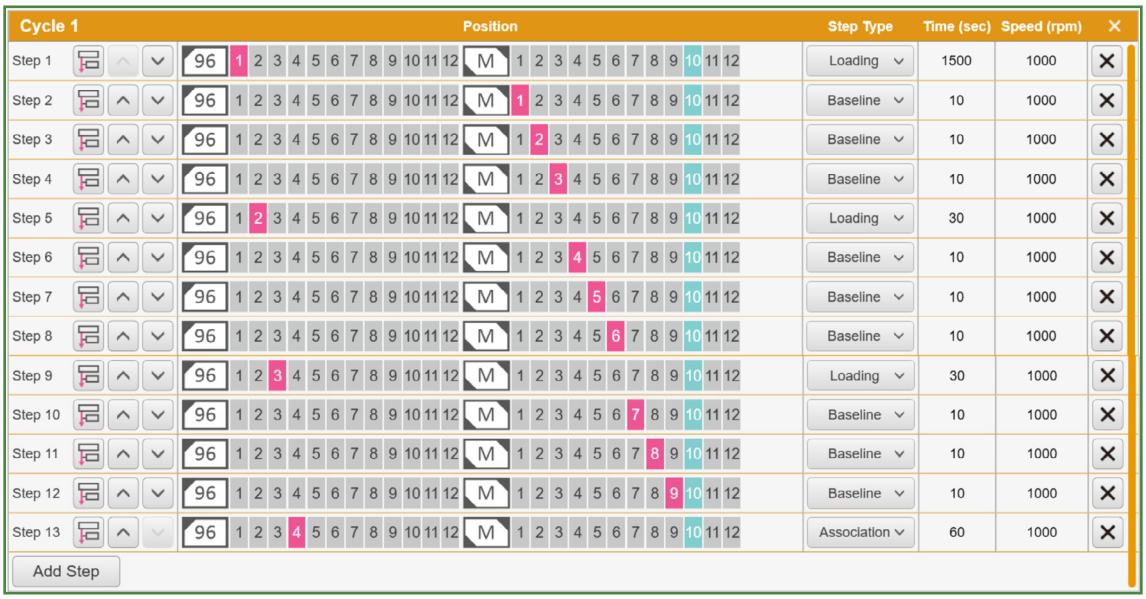
8. Under Plate Set Up, set up the Max Plate map.
   - Highlight columns 1-9 on the Max Plate and select “Wash”. Refer to image below.
   - Highlight columns 10 and select “Probe”.
     Note: Repeat step 8 for column 11 and column 12 for each consecutive assay.



9. Under the Assay Steps tab, set up the experimental cycle(s) as follows:
   - 1500 sec for sample
   - 10 sec for each wash step
   - 30 sec for Solution A
   - 30 sec for Solution B
   - 60 sec for Solution C
     Note: Refer to the image below to set up the step positions for Cycle 1.

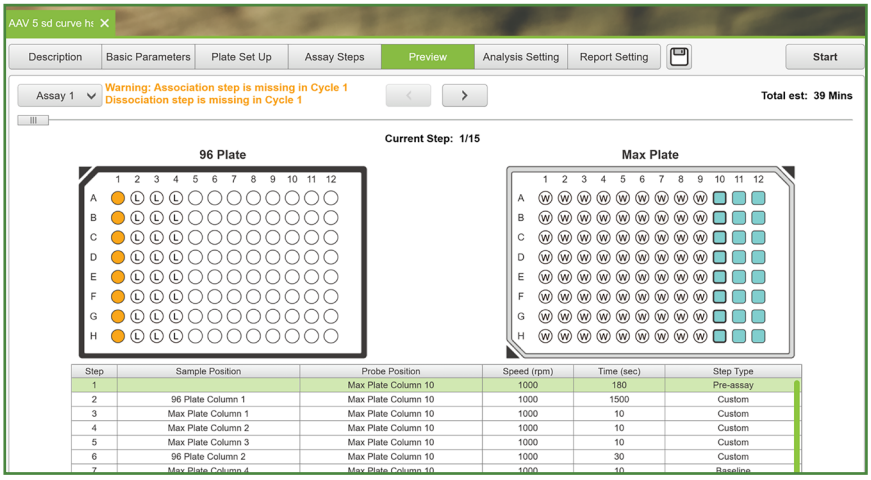
AAV Quantification Using Gator® High Sensitivity AAV Kit

Part No. 350003



10. Optional: Set up any consecutive assays. Skip to step 11 if not applicable.
    - For assay 2, click “add cycle”
    - Repeat step 9 to set up positions and steps
    - Highlight column 11 in blue for Cycle 2
    - For assay 3, click “add cycle”
    - Repeat step 9 to set up the positions and steps
    - Highlight column 12 in blue for Cycle 3

11. Under the Preview tab, confirm all of the step positions, times, and RPM. Refer to the image below.

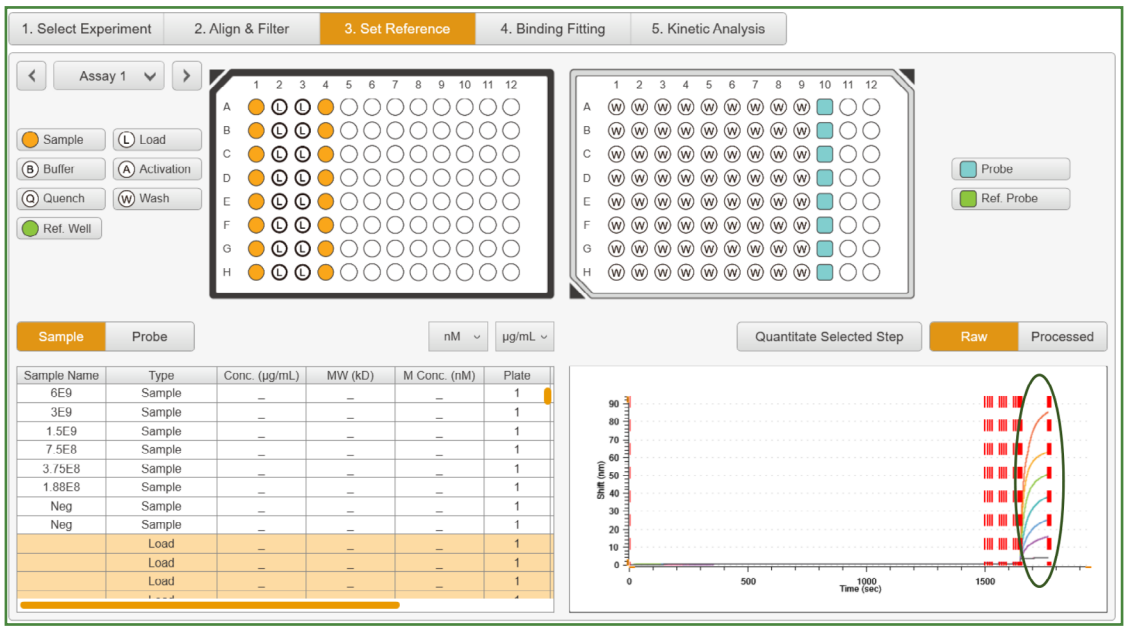


12. The assay will be completed in 36-99 minutes. After the assay is completed, the required data analysis can be performed.

Data Analysis

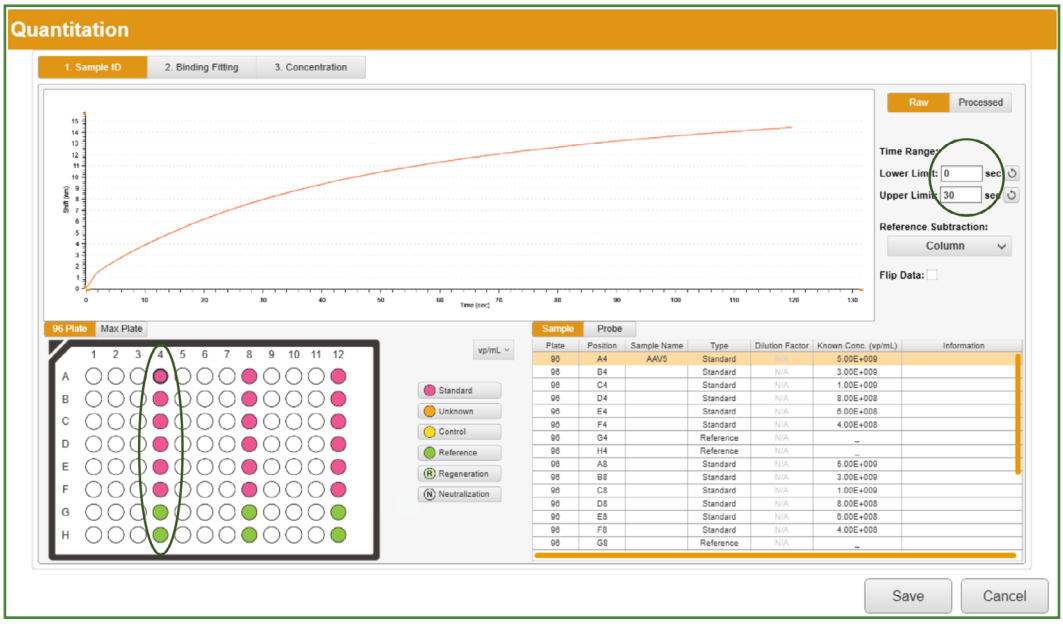
Standard Curve Analysis

1. Under the Results & Analysis tab, open the assay corresponding to a completed standard curve.
2. Click “New K Analysis 1”, select “Set Reference”, and then click on graph in the bottom right corner.



3. Select the “Quantitate Selected Step”.
   Note: The following message may appear: “Multiple assays are detected.” If “Yes” is selected, the other assays from the same run will be included in this standard curve. Select “No” if this is not applicable.
4. Under the Sample ID tab, adjust the following parameters to match those listed below (refer to the image for additional clarification):
   - Lower Limit: 0 sec
   - Upper Limit: 30 sec
   - Highlight the buffer blank samples and label as “Reference”
   - Reference subtraction: column
   - Highlight the remaining wells and label as “Standard”
   - Enter the known concentrations for the standards

AAV Quantification Using Gator® High Sensitivity AAV Kit Part No. 350003



5. Under the Binding Fitting tab, click “Parameters”. The following are the recommended parameters:
   - Equation: “REqilibriumOptimal”
   - Optimal: Leave as default value
   - Low Conc: Leave as default value
   - Click “Confirm” to proceed
   - Click “Binding Curve Fit” and allow a moment for the graph to recalibrate

6. Under the Concentration tab, click “Calculate Conc” and wait for the calculated concentration values to auto-populate in the table below.
7. Click “Save Std Curve” for future assays when working with the same AAV serotype.

AAV Quantification Using Gator® High Sensitivity AAV Kit Part No. 350003

Experimental Sample Analysis

1. Repeat steps 1-3. under Standard Curve Analysis.
2. Under the Sample ID tab, imput the following parameters:
   - Low Limit: 5 sec
   - Upper Limit: 35 sec
   - Reference Subtraction: None
   - All highlighted wells labeled as unknown
3. Under the Binding Fitting tab, select parameters. Recommended parameters are:
   - Equation: “REqulibriumOptimal”
   - Optimal: Leave as default value
   - Low Conc: Leave as default value
   - Click confirm to proceed
   - Select “Binding Curve Fit” and allow a moment for the graph to recalibrate
4. Under the Concentration tab, click “Parameters”.
5. Click “Load” to upload the desired serotype specific standard curve from the desktop.
6. Identify the preferred fitting model and select “Confirm”.
7. Select “Calculate Conc” and wait for the calculated concentration values to auto-populate in the table below.
8. Select the “Save” icon to save analysis report.

Notes:

Plate Set Up

- Ensure that that appropriate wells have been filled with the respective reagents and correspond correctly to the wells in the GatorOne software.
- Confirm that the pin placement in the Max Plate matches that in the GatorOne software.

Software

- If running multiple consecutive assays, change the probe column for every run under “Assay Set Up”. The regeneration error code will appear if the user fails to specify this information.
- Ensure that step 13 for every assay is labeled as “Association” under “Assay Set Up.” The data analysis will be unavailable if this step is labeled incorrectly.

AAV Quantification Using Gator® High Sensitivity AAV Kit Part No. 350003

Common Issues and Troubleshooting Tips

Issue	Potential Cause	Troubleshooting
Inconsistent signal shifts	Nonhomogeneous reagents	○ Vortex AAV stock solutions well (minimum of 30 seconds recommended) prior to preparing the AAV samples. ○ Mix Solutions A and B well before pipetting into the Black Plate wells
Insufficient signal separation from the negative control	Matrix interference contributing to an elevated background	○ If the sample is suspended in a matrix, consider diluting up to 1:1000-fold (the working concentration should remain within the serotype's dynamic range)
Minimal/no signal shift	Dead probe(s)	○ Rerun the assay with fresh probes