PROTOCOL

AAV Quantification Using Gator® AAVX Probes

SCOPE

This document provides a detailed protocol for running a quantification assay for adeno-associated virus (AAV) particles using Gator® AAVX probes and analyzing AAV quantitation data using the Gator® 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 utilized in viral gene therapy since they are non-integrating and non-immunogenic, reducing the risk for insertional mutagenesis in the host genome or an immune response. Gator® AAVX probes are highly useful for the quantification of different AAV serotypes using label-free bio-layer interferometry (BLI) technology. 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 probes are highly specific for AAV particles with an LoQ of $1x10^{9}$ viral particles (vp)/mL and an LoD of $5x10^{8}$ vp/mL. The dynamic range is over 4 orders of magnitude ($1x10^{9}$ – 10^{13} vp/mL). Furthermore, the probes can be regenerated up to 10 times using the Gator® Regen Buffer (Part No: 120008) with no loss of binding rate, resulting in accurate quantification of viral particles.

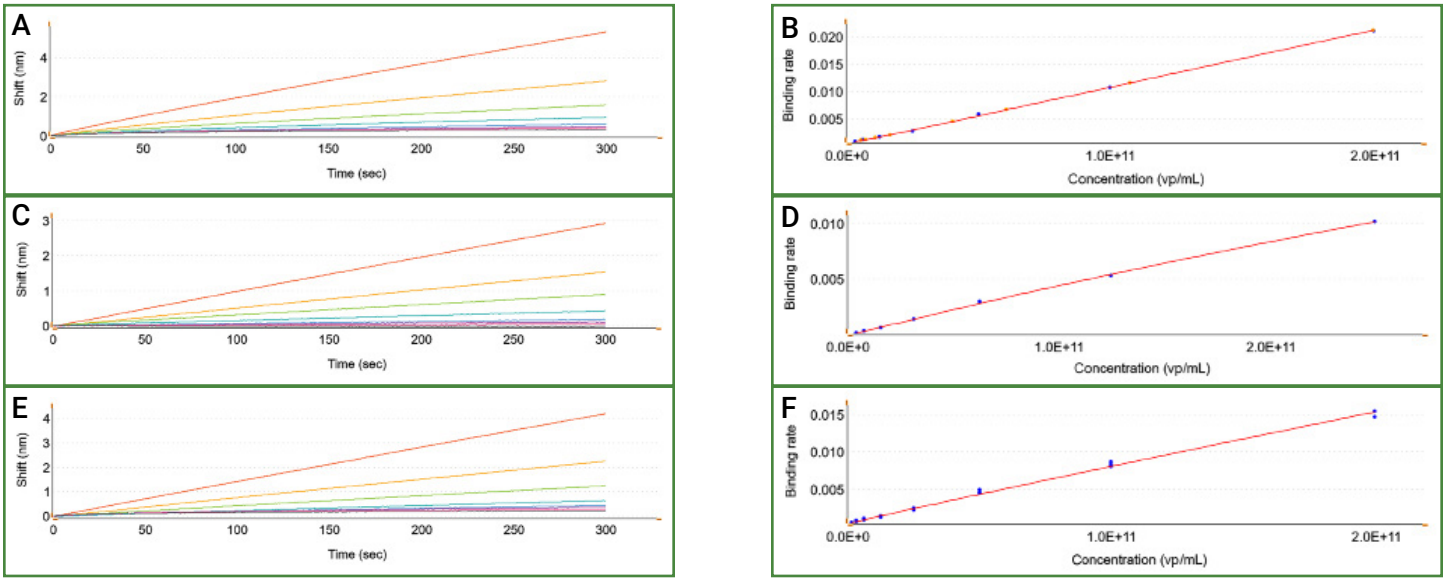

Figure 1. **(A)** Capture of AAV8 serotype at $3.12x10^{9}$ – $2x10^{11}$ vp/mL and **(B)** standard curve for AAV8. **(C)** Capture of AAV2 serotype at $3.9x10^{9}$ vp/mL – $2.5x10^{11}$ vp/mL and **(D)** standard curve for AAV2. **(E)** Capture of AAV4 serotype at $3.1x10^{9}$ vp/mL – $2x10^{11}$ vp/mL and **(F)** standard curve for AAV4. Each assay was performed using a 1:2 dilution series in Q Buffer with the capture of serotypes using Gator® AAVX probes. Standard curves were generated by the GatorOne software.

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MATERIALS REQUIRED

- Gator® AAVX Probe, Part No: 160017
- Gator® Quantitation (Q) Buffer, Part No: 120010
- Gator® Regen Buffer (No Salt), Part No: 120008
- Neutralization Buffer (Q Buffer or sample diluent if the samples are in another diluent such as media)
- Gator® Max Plate, Part No: 130062
- Black Plate
  Greiner Bio-One, Cat. No: 655209 (96-well) or Cat. No: 781209 (384-well)
  Gator® BLI 96-Flat Plate, Polypropylene, Part No: 130118-1PK or 130118-1CS
- Tweezer, Fisher Scientific, Cat No: 1495032

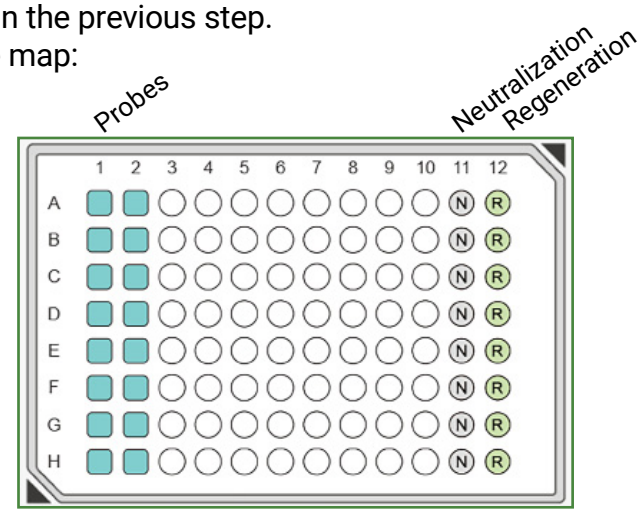
STORAGE

Store the AAVX probes at room temperature in their accompanying foil packaging with desiccants to avoid moisture. Under high humidity enviroments, storage in a dry cabinet is recommended.

AAVX QUANTIFICATION PROTOCOL

MAX PLATE SETUP

1. Add 250 µL/well of Q Buffer or sample diluent (if the samples are in another diluent such as media) to as many columns of the Max Plate as desired, depending on the number of samples being quantified. Leave columns 11 and 12 for regeneration reagents.
2. Add 250 µL/well of Q Buffer to column 11 of the Max Plate.
3. Add 250 µL/well of Regen Buffer to column 12 of the Max Plate.
4. Use the tweezer to pick out probes and place those in columns 1 and 2, into which 250 µL of Q Buffer has been added in the previous step.
   An example of the plate map:



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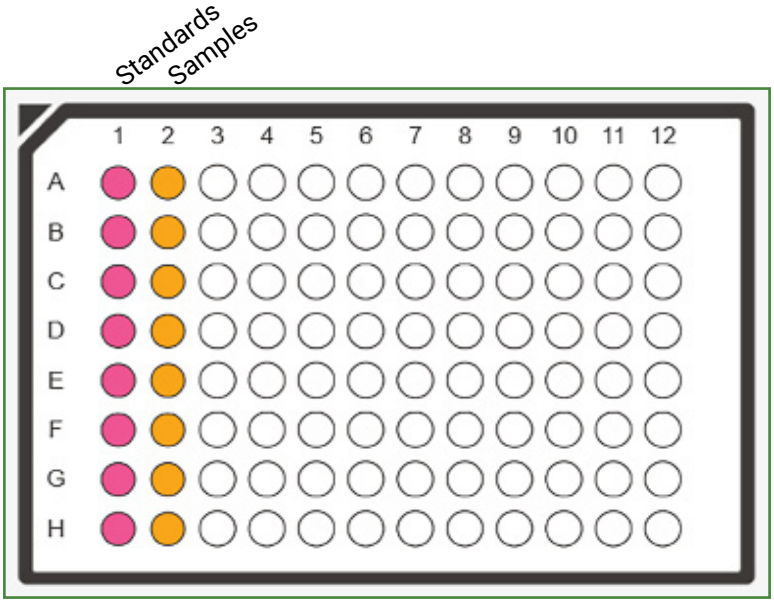
QUANTIFICATION PLATE SETUP

1. Add 200 µL/well of AAV standards to column 1 of the 96-well plate. Prepare the standards in the same diluent as the samples.
2. Add 200 µL/well of AAV samples into the remaining columns. For accurate results, test several dilutions of the samples to ensure that they fall within the detection range of the assay.
   An example of the plate map:



QUANTIFICATION ASSAY

1. On the Quick Start menu in the GatorOne software, select "Q" to start a quantification assay.
2. Click "Browse" to select the folder where the data will be saved in and rename the assay.
3. On the Gator® instrument, confirm that Shaker A is in the tilt position.
4. Place the quantification plate on Shaker A and the Max Plate on Shaker B.
5. Under "Description", input the required information.
6. Under "Basic Parameters", input the following:
   - Data Acquisition: 5 Hz
   - Shaker Setting: Tilt; Shaker A & B at 30°C
   - Pre-wet & Pre-Mix Setting: 600 sec
   - Shaker A & B at 0/1000 rpm

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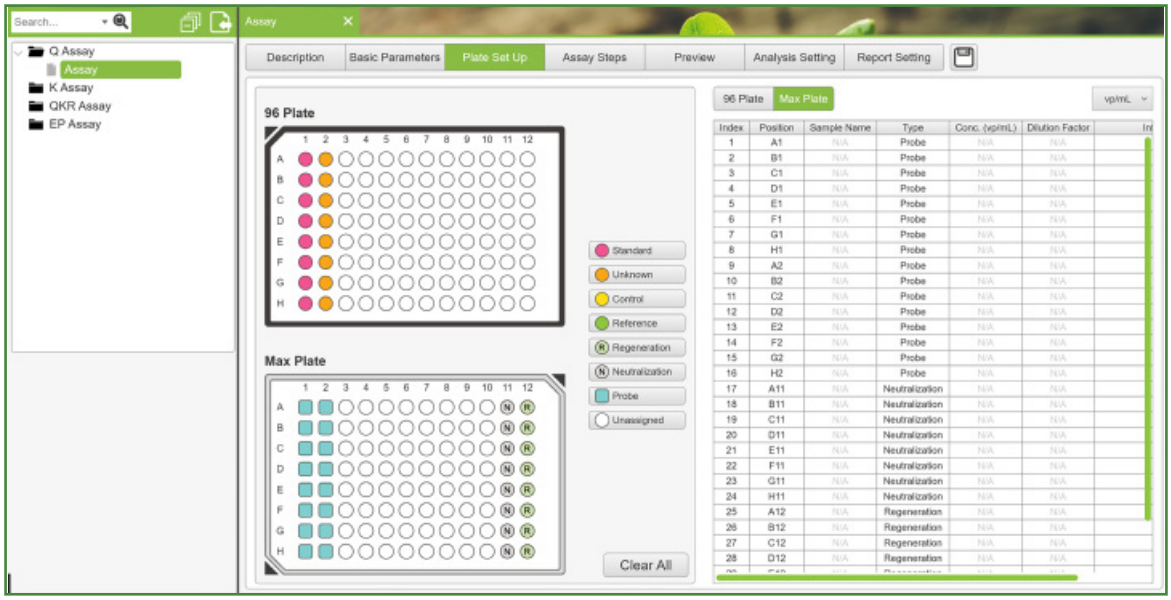
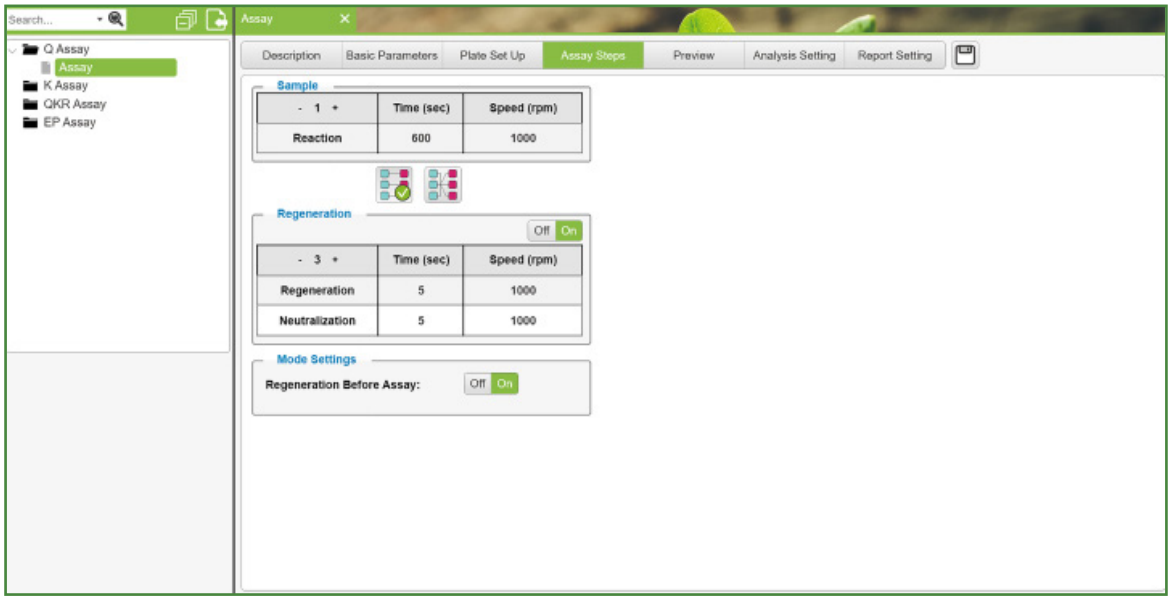
7. Under "Plate Set Up", set up the assay plate maps to indicate the standard and sample columns in the quantification plate and the buffer (Regen Buffer and Neutral Buffer) and probes in the Max Plate.



8. Under "Assay Setup", set up the assay with the following input as shown in the image below.
   Note: An initial Reaction Time of 300 sec at 1000 rpm is recommended, which can be increased as needed for optimization.
9. Under "Preview", check that all of the steps are correct and start the assay run.

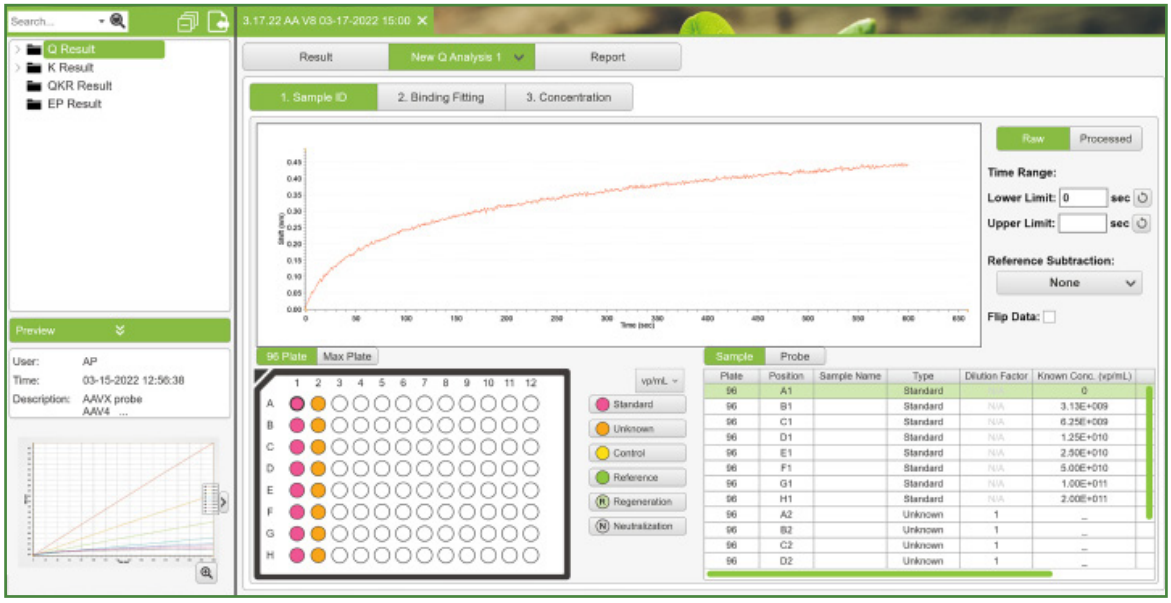


AAV Quantification Using Gator® AAVX Probes

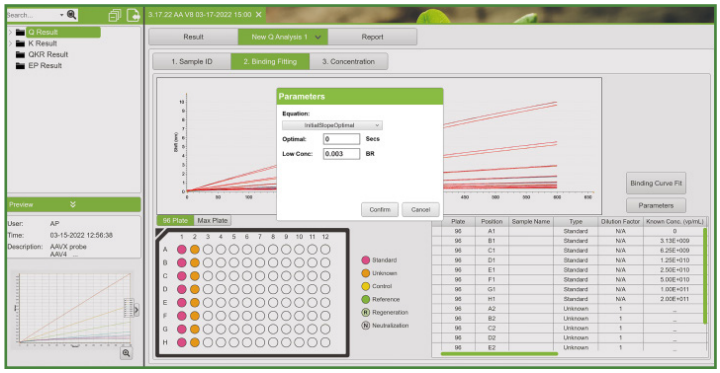
DATA ANALYSIS

After the assay is complete, the required data analysis should be performed.

1. Under "Q Results", go to "New Q Analysis".
2. Under "Sample ID", enter the concentrations for the standards in the column titled “Known Concentration”.



3. Under "Binding Fitting", click on "Parameters" and select "Initial Slope Optimal".
4. Click "Binding Curve Fit" to generate the binding rate graphs. This function will generate the binding rate data for all of the standards and unknown samples.



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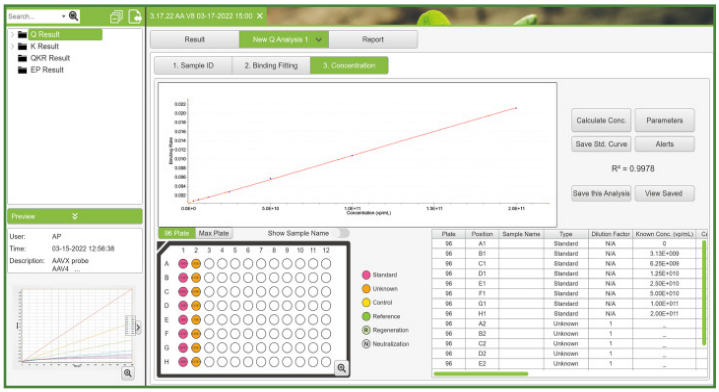
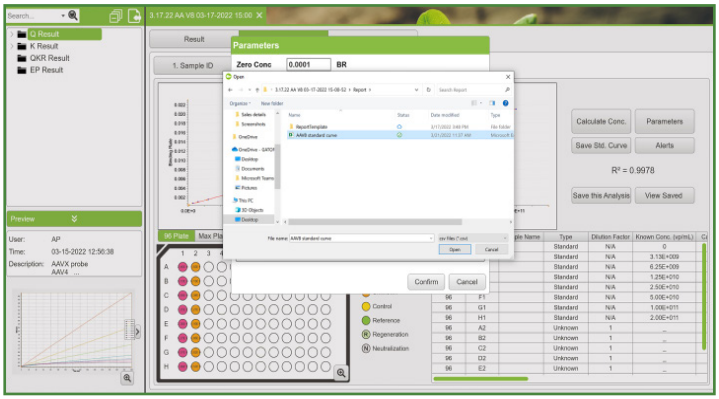
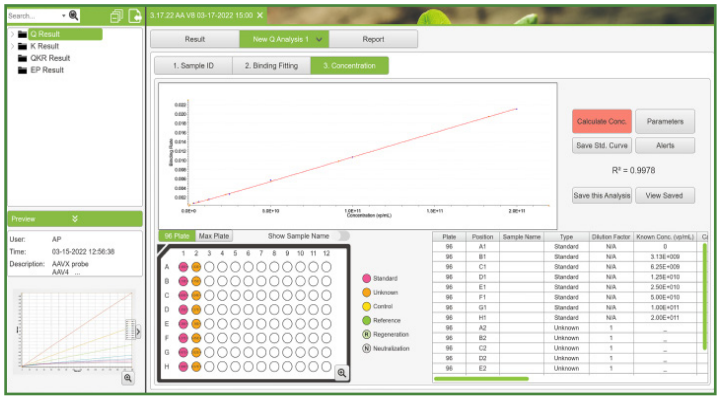
5. Under "Concentration", Click "Parameters" and select "FivePLRgressionWeightedY2".
6. Click "Confirm", followed by "Calculate Conc".
7. Click "Save Standard Curve" to save the standard curve for future anaysis. This function will save the standard curve as a ".csv" file, which can be loaded when running future analyses.



8. To load a previously saved standard curve, click "Parameters", followed by "Load", and select the file saved as standard curve. Hit "Confirm".
9. Proceed by clicking "Calculate Conc." to calculate the concentrations of the unknown samples.





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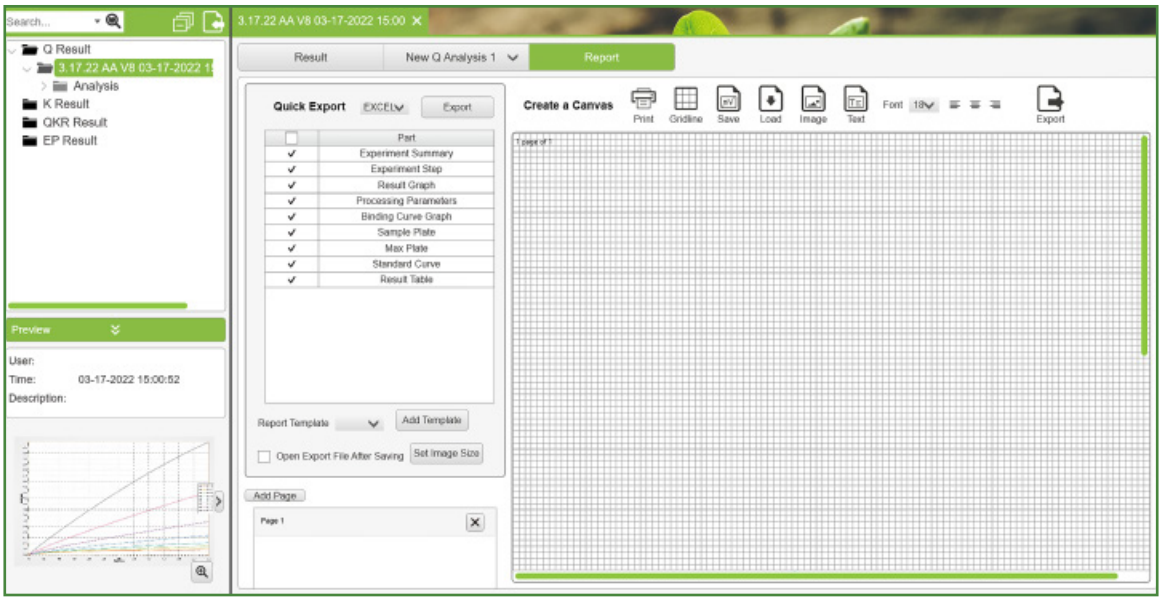
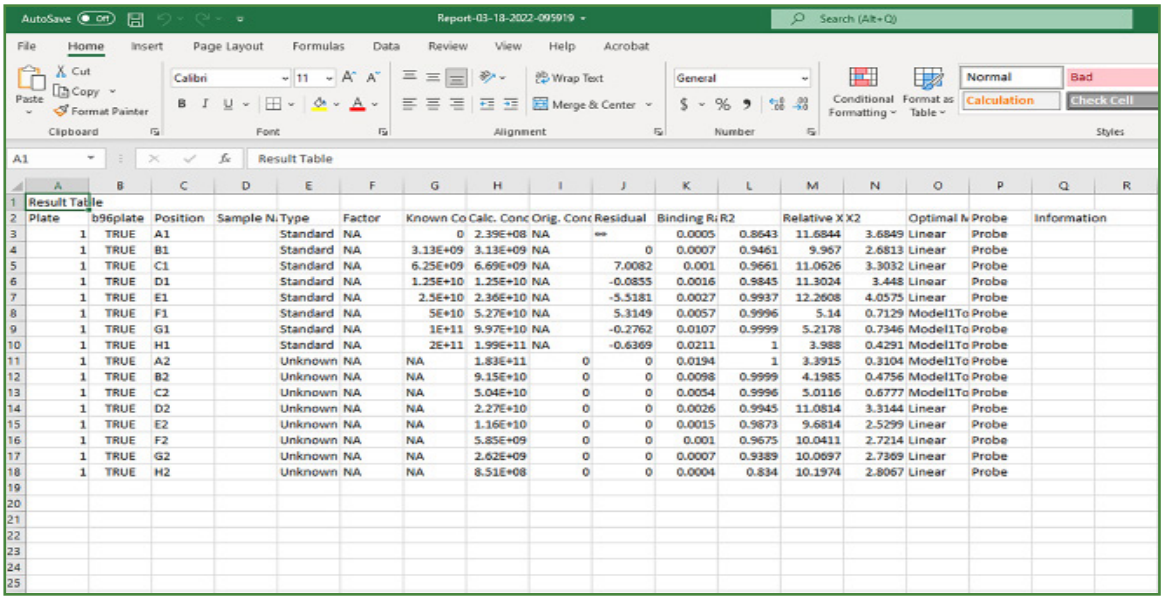
10. Under Report, export the data as an Excel file.



An example of a data report exported as an Excel file:



Result Table																	
Plate	b96plate	Position	Sample Na	Type	Factor	Known Co	Calc. Conc	Orig. Conc	Residual	Binding Ra	R2	Relative X	X2	Optimal M	Probe	Information	
1	TRUE	A1		Standard	NA	0	2.39E+08	NA	∞	0.0005	0.8643	11.6844	3.6849	Linear	Probe		
1	TRUE	B1		Standard	NA	3.13E+09	3.13E+09	NA	0	0.0007	0.9461	9.957	2.6813	Linear	Probe		
1	TRUE	C1		Standard	NA	6.25E+09	6.69E+09	NA	7.0082	0.001	0.9661	11.0626	3.3032	Linear	Probe		
1	TRUE	D1		Standard	NA	1.25E+10	1.25E+10	NA	-0.0855	0.0016	0.9845	11.3024	3.448	Linear	Probe		
1	TRUE	E1		Standard	NA	2.5E+10	2.36E+10	NA	-5.5181	0.0027	0.9937	12.2608	4.0575	Linear	Probe		
1	TRUE	F1		Standard	NA	5E+10	5.27E+10	NA	5.3149	0.0057	0.9996	5.14	0.7129	Model1To	Probe		
1	TRUE	G1		Standard	NA	1E+11	9.97E+10	NA	-0.2762	0.0107	0.9999	5.2178	0.7346	Model1To	Probe		
1	TRUE	H1		Standard	NA	2E+11	1.99E+11	NA	-0.6369	0.0211	1	3.988	0.4291	Model1To	Probe		
1	TRUE	A2		Unknown	NA	NA	1.83E+11	0	0	0.0194	1	3.3915	0.3104	Model1To	Probe		
1	TRUE	B2		Unknown	NA	NA	9.15E+10	0	0	0.0098	0.9999	4.1985	0.4756	Model1To	Probe		
1	TRUE	C2		Unknown	NA	NA	5.04E+10	0	0	0.0054	0.9996	5.0116	0.6777	Model1To	Probe		
1	TRUE	D2		Unknown	NA	NA	2.27E+10	0	0	0.0026	0.9945	11.0814	3.3144	Linear	Probe		
1	TRUE	E2		Unknown	NA	NA	1.16E+10	0	0	0.0015	0.9873	9.6814	2.5299	Linear	Probe		
1	TRUE	F2		Unknown	NA	NA	5.85E+09	0	0	0.001	0.9675	10.0411	2.7214	Linear	Probe		
1	TRUE	G2		Unknown	NA	NA	2.62E+09	0	0	0.0007	0.9389	10.0697	2.7369	Linear	Probe		
1	TRUE	H2		Unknown	NA	NA	8.51E+08	0	0	0.0004	0.834	10.1974	2.8067	Linear	Probe		

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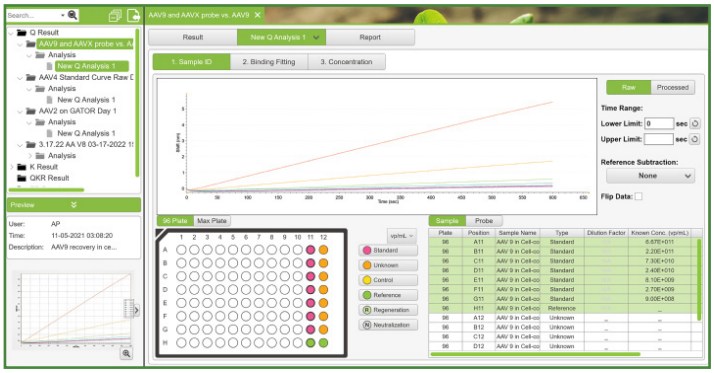
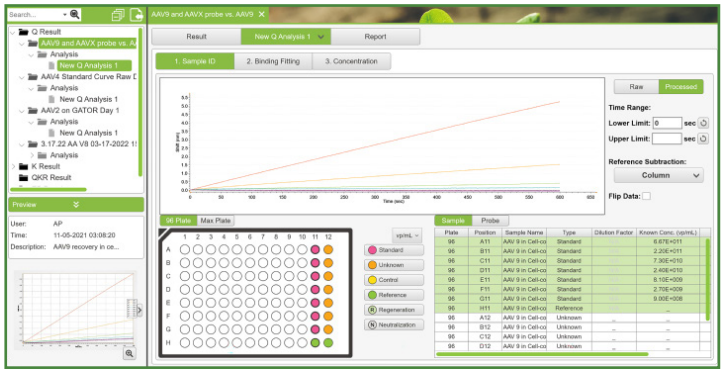
SAMPLES IN MEDIA

AAVX probes can be used to quantify AAV particles in media. Prepare AAV standards using the same media/diluent that the samples are in. If the sample media is being diluted with Q Buffer (or any other diluent), the standards should be diluted using the same diluent to enable direct comparison of the samples to the standards.

DATA ANALYSIS

1. Under "Q Results", proceed to "New Q Analysis".
2. Under "Sample ID", enter the concentrations for the standards in the column titled "Known Concentration".
   - In the 96-well plate map, highlight the wells with blank media as the Reference.
   - Under "Reference Subtraction", enter "Column", "Row", or "All" depending on whether the samples are in the same column, in the same row, or all of the samples in the plates are to be reference subtracted.
3. Click "Processed" to display the reference subtracted data.





4. Under "Binding Fitting", click "Parameters" and select "Initial Slope Optimal".
5. Click "Binding Curve Fit" to generate the binding rate graphs. This function will generate the binding rate data for all of the standards and unknown samples.
6. Under "Concentration", click "Parameters", and select "FivePLRgressionWeightedY2".
7. Select "Confirm" and click "Calculate Conc".

   If a previously saved standard curve is being loaded, ensure that the standard curve is in the same media/diluent as the samples. Any difference will lead to erroneous results. If unsure, run a new standard curve.
8. The data can be exported as previously described.

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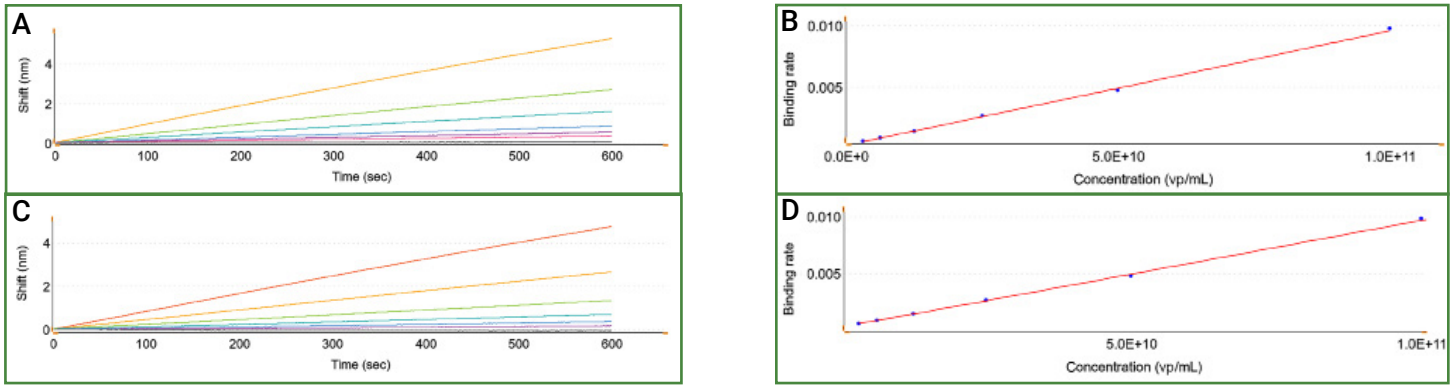

Figure 2. **(A)** Capture of AAV9 serotype and **(B)** standard curve for AAV9 in Q Buffer. **(C)** Capture of AAV9 serotype and **(D)** standard curve for AAV9 in CHO cell media. The concentration range is 3.12x10⁹ vp/mL to 1x10¹¹ vp/mL for both assays, and performed with a 1:2 dilution series using Gator® AAVX probes. Standard curves were generated by the GatorOne software.

COMMON ISSUES AND TROUBLESHOOTING

Issue	Potential Cause	Troubleshooting
No binding signal in samples	AVV concentration too low	Try increasing the reaction time (e.g., >120 sec)
Binding signal in samples lower than expected	Interference or matrix effects from sample diluent / media	Make sure the standards and samples are run in the same diluent
Binding rates too fast and clustered together	AAV concentration in samples too high	Dilute sample and try different dilutions
Standard curve nm shift too low	Standard concentration too low	Recheck standard concentration and make sure it is in the range $1x10^9 - 1x10^{13}$
	Standards degraded	Prepare fresh AAV stock and prepare fresh standards by serial dilution

CONCLUSION

The Gator® AAVX probe, paried with the Gator® instrument, is a useful tool for the quantification of different serotypes of AAV viral particles in cell lysates and cell culture supernatants. It offers clear advantages over traditional methods (e.g., qPCR, ddPCR, and ELISA) such as fast turnaround assay time, little hands-on activity, and convenience due to the use of few reagents. The probes are also highly cost effective as they can be regenerated up to 10 times, thus enabling the user to get 1000 assays from a tray of 96 probes.

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