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.



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

- 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 $\mu L/well$ 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.
- 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:





Gator Bio, Inc. 2455 Faber Place, Palo Alto, CA 94303, USA • +1 855 208 0743 • info@gatorbio.com • GatorBio.com © 2022 Gator Bio, Inc. All rights reserved. Gator is a registered trademark of Gator Bio, Inc. P017_6-2022

QUANTIFICATION PLATE SETUP

- 1. Add 200 μ L/well of AAV standards to column 1 of the 96-well plate. Prepare the standards in the same diluent as the samples.
- 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



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.

K Assay QKR Assay: EP Assay: 96 Plate 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 7 8 9 10 11 12 14	Assay Assay	Description Basic Parameters Plate Set Up Ad	ssay Steps Prev	iew	Analysis	Setting Rep	oort Setting			
EP Assay 1 2	: Assay XKR Assay	96 Plate		96 P	late Max	Plate				vp/mL
1 2 3 4 5 6 7 8 9 10 11 12 A 0 <th>P Assay</th> <th></th> <th></th> <th>Index</th> <th>Position</th> <th>Sample Name</th> <th>Type</th> <th>Conc. (vp/mL)</th> <th>Dilution Factor</th> <th>-</th>	P Assay			Index	Position	Sample Name	Type	Conc. (vp/mL)	Dilution Factor	-
A O		1 2 3 4 5 8 7 8 9 10 11 12		1	A1	NUA	Probe	NIA	NM	-
B O				2	81	NUA	Probe			
4 D1 NA Picke NA <				3	C1	NUA.	Probe			
C C				- 4	D1	N04	Probe			
D O				5	E1	34.04	Probe			
E Image: Construction of the second of t				6	F1	NUA	Probe			
- -				7	G1	NUA	Probe			
F Q			Standard	8	H1	NVA.	Probe			
G O			Automa 1	9	A2	NUA	Probe			
H Image: Construction of the second seco		III ⊂ ●●○○○○○○○○○○ I	Unknown	10	82	1N.0A	Probe			
Max Plate ¹²		LL * ••••••••••	Control	11	C2	NUA	Probe			
Image: Constraint of the second sec				12	D2	N.04	Probe			
Max Plate Image: Second S			Reference	13	E2	NUA.	Probe			
Max Plate 15 02 900			(R) Regeneration	14	F2	51.04	Probe			
1 2 3 4 5 6 7 8 9 10 11 12 14 Probe 140 Not Not Not A 0 0 0 0 0 0 0 16 H2 104 Probe 105 Not Not B 0 0 0 0 0 0 0 16 H2 104 Not Not <t< td=""><td></td><td>Max Plate</td><td>C</td><td>15</td><td>G2</td><td>NUA -</td><td>Probe</td><td></td><td></td><td></td></t<>		Max Plate	C	15	G2	NUA -	Probe			
1 2 3 4 5 6 7 8 9 10 11 12 A Image: Constraint of the state			(N) Neutralization	16	H2	NUA	Probe			
A Image: Constraint of the second		1 2 3 4 5 6 7 8 9 10 11 12	Poste .	17	A11	NUA	Neutralization			
B O O W Image: grad 19 C11 NUA NUA NUA C O O W W Image: grad 20 D11 NUA NUA NUA 21 E11 RUA Neutralization NUA RUA RUA 22 F11 RUA Neutralization NUA RUA 23 G111 AUA Neutralization NUA RUA 24 H11 RUA Regeneration NUA RUA 24 H11 RUA Rua RUA RUA 24 H11 <t< td=""><td></td><td></td><td>Prove</td><td>18</td><td>B11</td><td>NU4</td><td>Neutralization</td><td></td><td></td><td></td></t<>			Prove	18	B11	NU4	Neutralization			
20 D11 50/A Nutritization 18/A 70/A 21 E11 50/A Nutritization 18/A Nutritization 18/A Nutritization 10 D O O 0 0 0 0 18/A Nutritization 18/A Nutritization 11 Static Static Static Static Static 18/A Nutritization 18/A Nutritization 12 G11 Nutritization			Unassigned	19	C11	N04	Neutralization			
C Image: Comparison of the comparison				20	D11	805	Neutralization			
p 22 F11 VUA Neutralization VUA VUA 23 G11 VUA Neutralization VUA VUA 24 H11 VUA Neutralization VUA NUA 24 H11 VUA Neutralization VUA NUA 25 A12 FUA Regeneration VUA NUA 26 A12 FUA Regeneration VUA NUA 26 A12 FUA Regeneration VUA VUA 27 C12 VUA Regeneration VUA VUA 27 C12 VUA Regeneration VUA VUA				21	E11	NUA	Neutralization			
E O O O NA NA NA F O O O NA NA NA NA G O O O NA NA NA NA G O O O NA NA NA NA G O O O NA NA NA NA H O O O NA NA NA NA H O O O NA NA NA NA H O O NA NA NA NA				22	F11	NU4	Neutralization			
P O				23	G11	NUA .	Neutralization			
P O O O W (B) 25 A12 VUA Regressriation VUA VUA G O O O W (B) 28 B12 NUA Regressriation NUA NUA H O O O W (B) 27 G12 NUA Regressriation NUA NUA 24 D O O W (B) D D NUA NUA				24	H11	NUA	Neutralization			
G B12 NA Regeneration NA NA H B O O B B 24 B12 NA Regeneration NA NA H B O O O B B 24 B12 NA Regeneration NA NA				25	A12	14.04	Regeneration			
H Image: Constraint of the second secon				28	B12	NUA :	Regeneration			
28 D12 NVA Becommendian NVA NVA				27	C12	NUA	Regeneration			
Clear All co ote requirement			Clear All	28	D12	N.04	Regeneration			

- 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.

Search 🔹 🔍 🗐 💽	Assay X	and the second second			Street Street Street
Q Assay	Description Basic Pa	rameters Plate Set Up	Assay Steps Preview	Analysis Setting Report Setting	
K Assay	- Sample				
CKR Assay	- 1 +	Time (sec) Speed (rpm)			
	Reaction	600 1000			
	Demonstration	3 34			
	- Regeneration	Off	On		
	- 3 *	Time (sec) Speed (rpm)			
	Regeneration	5 1000			
	Neutralization	5 1000			
	Mode Settings Regeneration Before As	ssay: Off On			

Gator Bio, Inc. 2455 Faber Place, Palo Alto, CA 94303, USA • +1 855 208 0743 • info@gatorbio.com • GatorBio.com © 2022 Gator Bio, Inc. All rights reserved. Gator is a registered trademark of Gator Bio, Inc. P017_6-2022

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".

Search • 🍳 🗐 🕒	3.17.22 AA V8 03-17-2022 15:00 X	100 M		-	1	-		
> Q Result	Result New Q Analysis 1 🗸	Report						
GKR Result	1. Sample ID 2. Binding Fitting 3. Co	oncentration						
Preview 😽	0.49 0.49 0.39 0.39 0.39 0.39 0.39 0.39 0.39 0.3	20 20 3m jaci	400 400 Samele	900 Probe	519	856 0	Time Ra Lower L Upper L Referen	w Processed inge: imit: imit: see imit: see imit: None a:
Time: 03-15-2022 12-58-38		unimi ~	Plate	Position	Sample Name	Type	Dilution Factor	Known Conc. (vp/mL)
Description: AMX note		spine -	96	A1		Standard		0
AAV4		Standard	96	B1		Standard	NVA	3.13E+009
	∎ ●●00000000000	O Unknown	96	C1		Standard	NIA	6.25E+009
	■ • • • • • • • • • • • • • • • • • • •	Control	96	E1		Standard	NUA.	2.50E+010
		Como	96	F1		Standard	NUA	5.00E+010
		Reference	96	G1		Standard	NVA	1.00E+011
É S		(R) Regeneration	96	H1		Standard	NIA	2.00E+011
	 		96	A2		Unknown	1	
	s 000000000000000000000000000000000000	(N) Neutralization	96	82		Unknown	1	-
			96	C2	-	Unknown	1	-
			96	D2		Unknown	1	-
<u> </u>	(C	-						

- 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.



Gator Bio, Inc. 2455 Faber Place, Palo Alto, CA 94303, USA • +1 855 208 0743 • info@gatorbio.com • GatorBio.com © 2022 Gator Bio, Inc. All rights reserved. Gator is a registered trademark of Gator Bio, Inc. P017_6-2022



- 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 analysis. 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.





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

Search • 🔍 🗇 🕞	3.17.22 AA VB 03-17-2022 15:00 ×
3.17.22 AA V8 03-17-2022 1	Result New Q Analysis 1 V Report
> 🖬 Analysis K Result OKR Result	Quick Export Excelv Export Create a Canvas Image Image Image Text Fort Text
EP Result	Part
- Cr Hoodin	Experiment Summary
	V Experiment Step
	V Result Graph
	V Processing Parameters
	V Binding Curve Graph
	V Sample Plate
	V Max Plate
	V Standard Corve
Preview S	
User:	
Time: 03-17-2022 15:00:52	
Description:	
	Report Template V Add Template
	Open Export File After Saving Set Image Size
	Add Page
	Page 1

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

A	itoSave 🤅	01	圁	5-6				Repo	t-03-18-202	2-095919 -				€ Searc	h (Alt+Q)				
File	Ho	me	Inse	rt Pag	ge Layout	Formula	is Data	a Review	View	Help	Acrobat	1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1		303		3			
fi	X Cu	t		Calibri		~ 11 ~	A' A'	= = =	æ.	🕸 Wrap T	esct	General		-			Normal	Bad	
Pas	te Co	ру ~		BI	U + H	- A.	A	= = =		Harra .	& Cantar v	¢ . 9		0 00 Co	nditional	Format as	Calculatio	n Check	Cell
~	S For	mat Pa	iinter		8.10		-		1 12 12	Meige	or center -		0 / 0	For	matting ~	Table ~			
	Clipboa	rd	1	a	Fon	c.	151		Aligne	ent		5 N	umber	5				Stj	yles
A1		-		~ ~	fx Ret	sult Table													
	A	6		c	D	E	F	G	н	1	1	K	L	M	N	0	P	Q	R
2 5	lesult Tat	h96o	ate	Desition	Sample N	Tune	Factor	Known Co	Calc Con	Oria Con	Recidual	Rinding R: I	22	Relative V	12	Ontimal	Probe	Information	
3	1010	TR	IF	41	Jampie re	Standard	NA	0	2 395+08	NA NA		0.0005	0.8543	11 6844	3 6849	Linear	Probe	and the second	
4	1	TR	JE	81		Standard	NA	3.13E+09	3.13E+09	NA	0	0.0007	0.9461	9.967	2.6813	Linear	Probe		
5	1	TR	JE	C1		Standard	NA	6.25E+09	6.69E+09	NA	7.0082	0.001	0.9661	11.0626	3.3032	Linear	Probe		
6	1	TR	JE	D1		Standard	NA	1.25E+10	1.25E+10	NA	-0.0855	0.0016	0.9845	11.3024	3.448	Linear	Probe		
7	1	TR	JE	E1		Standard	NA	2.5E+10	2.36E+10	NA	-5.5181	0.0027	0.9937	12.2608	4.0575	Linear	Probe		
8	1	TR	JE	F1		Standard	NA	5E+10	5.27E+10	NA	5.3149	0.0057	0.9996	5.14	0.7129	Model1T	o Probe		
9	1	TR	JE	G1		Standard	NA	1E+11	9.97E+10	NA	-0.2762	0.0107	0.9999	5.2178	0.7346	Model1T	o Probe		
10	1	TR	JE	H1		Standard	NA	2E+11	1.99E+11	NA	-0.6369	0.0211	1	3.988	0.4291	Model1T	o Probe		
11	1	TR	JE	A2		Unknown	NA	NA	1.83E+11	0	0	0.0194	1	3.3915	0.3104	Model1T	o Probe		
12	1	TR	JE	B2		Unknown	NA	NA	9.15E+10	0	0	0.0098	0.9999	4.1985	0.4756	Model1T	o Probe		
13	1	TR	JE	C2		Unknown	NA	NA	5.04E+10	0	0	0.0054	0.9996	5.0116	0.6777	Model1T	o Probe		
14	1	TR	JE	D2		Unknown	NA	NA	2.27E+10	0	0	0.0026	0.9945	11.0814	3.3144	Linear	Probe		
15	1	TR	JE	E2		Unknown	NA	NA	1.16E+10	0	0	0.0015	0.9873	9.6814	2.5299	Linear	Probe		
16	1	TR	JE	F2		Unknown	NA	NA	5.85E+09	C	0	0.001	0.9675	10.0411	2.7214	Linear	Probe		
17	1	TR	JE	G2		Unknown	NA	NA	2.62E+09	0	0	0.0007	0.9389	10.0697	2,7369	Linear	Probe		
18	1	TR	JE	H2		Unknown	NA	NA	8.51E+08	0	0	0.0004	0.834	10.1974	2.8067	Linear	Probe		
19																			
20																			
21																			
22																			
23																			
24																			
25																			

Gator Bio, Inc. 2455 Faber Place, Palo Alto, CA 94303, USA • +1 855 208 0743 • info@gatorbio.com • GatorBio.com © 2022 Gator Bio, Inc. All rights reserved. Gator is a registered trademark of Gator Bio, Inc. P017_6-2022

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.

Search 🔹 🌒 🕞	AAV9 and AAVX probe vs. AAV9 X		Search 🔍 🗊 💽	AAV9 and AAVX probe vs. AAV9 ×		and the second second
 Q Result AAV9 and AAVX probe vs. Ar 	Result New Q Analysis 1 👻 Report		Q Result ANV9 and AAVX probe vs. Ar	Result New Q Analysis 1 👻	Report	
Anatysis New Q Analysis 1	1. Sample D 2. Binding Fitting 3. Concentration		 Analysis New Q Analysis 1 	1. Semple ID 2. Binding Fitting 3. Conce	intration	
AAV4 Standard Curve Raw L Analysis New Q Analysis 1		Raw Processed	Anitysis New Q Analysis 1	3.5		Raw Processed
AAV2 on GATOR Day 1 Analysis	5	Time Range:	AAV2 on GATOR Day 1 The Analysis	50 45 40		Time Range: Lower Limit: 0 sec 신
New Q Analysis 1	had approximately a second sec	Upper Limit: sec 🛈	New Q Analysis 1 3.17.22 AA V8 03-17-2022 1!	30 30 25		Upper Limit: sec 🔘
> E Analysis		Reference Subtraction:	> 📷 Analysis > 🖿 K Result	20 US US		Reference Subtraction:
GRR Result	9 0 56 150 150 268 208 308260	400 403 500 50 800 800 Filip Data:		0.5 0 50 100 150 290 250		Flip Data:
Preview ¥ User: AP	96 Plate Max Plate	Sample Probe	Preview S	96 Plate Max Plate	Sample Probe	
Time: 11-05-2021 03:08:20 Description: AAV9 recovery in ce	1 2 3 4 5 6 7 8 9 10 11 12 yabeL -	Plate Position Sample Name Type Dilution Factor Known Core. (spimL) 96 A11 AV/9 in Col-oo Stendard 6.671(-011 96 B11 AV/9 in Col-oo Stendard 2.205(-015)	Time: 11-05-2021 03:08:20 Description: AAV9 recovery in ce		vpint, Plate Position Sample Nerre Type D 96 A11 AW 9 in Cel-co Standard 96 B11 AW 9 in Cel-co Standard 96 B11 AW 9 in Cel-co Standard	284601 Factor Known Conc. (spin6.) 6.67E+011 2.20E+011
		96 C11 AAV 9 in Cell-co Standard 7.30E+010 96 D11 AAV 9 in Cell-co Standard 2.40E+010 96 D11 AAV 9 in Cell-co Standard 2.40E+010		• 00000000000	Unknown 96 C11 AW 9 in Cell co Standard 98 D11 AW 9 in Cell co Standard Co Standard Co Co	7.30E+010 2.40E+010
		96 E11 Add 9 in Celeco standard 6.10E+009 96 F11 Add 9 in Celeco Standard 2.70E+009 96 G11 Add 9 in Celeco Standard 9.00E+008		· 00000000000	Control 90 E11 AW 9 in Cell-co Standard 96 C11 AW 9 in Cell-co Standard 96 C11 AW 9 in Cell-co Standard	2.70E+009 9.00E+008
1 1	F OOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOO	96 H11 AW/9 in Cel-co Reference	18	F 000000000000	(B) Regeneration 96 H11 AW/ 9 in Cell-co Reference 96 A12 AAV 9 in Cell-co Ustream 96 A12 AAV 9 in Cell-co Ustream 96 B12 AAV 9 in Cell-co Ustream Ustream	2 2 1
	* 00000000000	96 C12 AW/9 in Cel-co Unknown		+ 000000000000	96 C12 AAV 9 in Cell-co Utsknown 96 D12 AAV 9 in Cell-co Utsknown	
N.				10		

- 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.





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 3.12x10° 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	Standard concentration too low	Recheck standard concentration and make sure it is in the range $1 \times 10^9 - 1 \times 10^{13}$			
too low	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.