APPLICATION NOTE

Comparison of Gator® BLI and Progen ELISA for Quantitation of AAV Serotypes

INTRODUCTION

Determination of total capsid titer is one of the critical quality attributes for AAVs used in gene therapy.

This application note presents quantitation of AAV serotype 2, 5, 8 and 9 using Gator® Prime system and Gator® AAVX probes and compares the performance with Progen Xpress ELISA assay for the same serotypes.

ELISA was run on SpectraMax iD5, Molecular Devices. The AAV serotype standards were purchased from Virovek, Newark, CA.

GATOR® AAVX PROBE FEATURES

- Total capsid quantitation for serotypes AAV1-10
- Dynamic range up to 1E+14 vp/mL (for most serotypes)
- Less than 30 min analysis time
- LOD ~ 1E+09 vp/mL
- Crude sample tolerant
- Stable over broad pH range
- Cost effective
- Easy to use with minimal hands-on time

QUANTITATION PRINCIPLE AND WORKFLOW

- Gator® probe uses CapSelect™ AAVX nanobody as a ligand to enable direct measurement
- Samples containing AAV particles are pipetted into the 96-well plate and captured on the probe
- The total virus capsid concentration determined using rate of binding of the AAV serotype of interest to the probe
- Different AAV serotypes bind at different rates
- Gator® software calculates the binding rates from standards with known concentrations to generate a standard curve

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Comparison of Gator® BLI and Progen ELISA for Quantitation of AAV Serotype 9

Method: The performance of Gator® BLI platform for quantitation of various AAV serotypes was evaluated using an established and relatively commonly used Xpress ELISA kit.

The Virovek AAV2, 5, 8 and 9 standards were at a highest concentration of 1E+11, 1E+11, 2E+11 and 2E+11 vp/mL, respectively. For ELISA, the stocks were diluted 100x, 10x, 400x and 300x, respectively, to bring into the ELISA assay.

AAV9 CONCENTRATION- GATOR® AAVX PROBES VS AAV9 ELISA

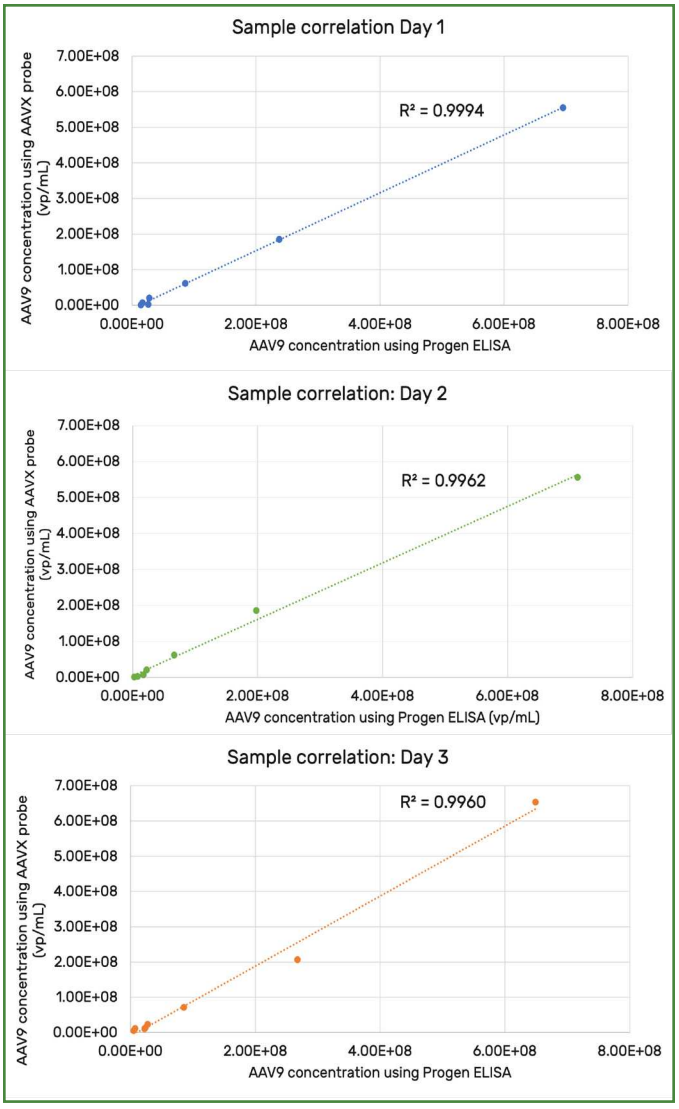

Figure 1: Correlation between Gator® and ELISA assay AAV9

The ELISA kit performance was verified on SpectraMax iD5. As can be seen in Figure 1, the kit shows good inter-day reproducibility over 3 days. Also, as can be seen in Figure 2, the ELISA measurements were linear as specified for the kit.

VERIFICATION OF AAV9 ELISA LINEARITY

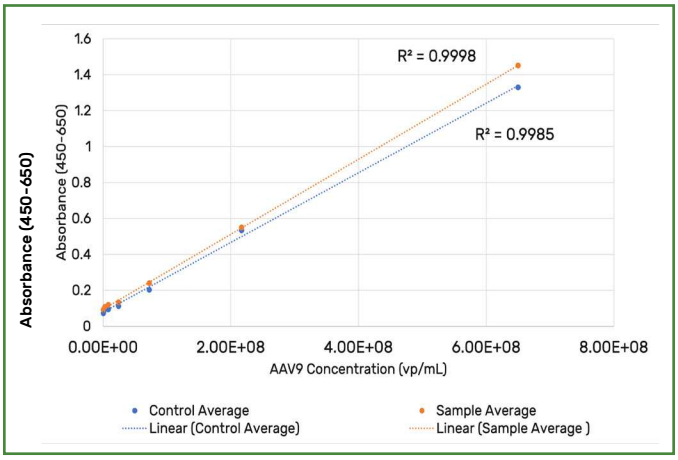

Figure 2: Linearity in the dynamic range specified for the kit.

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Comparison of Gator® BLI and Progen ELISA for Quantitation of AAV Serotype 2

% ERROR OF ELISA AND GATOR® FOR AAV2

Known vp/mL	Progen ELISA vp/mL	Progen ELISA % Error	Gator vp/mL	Gator % Error
1.00E+09	1.47E+09	47%	1.17E+09	17%
5.00E+08	9.06E+08	81%	6.12E+08	22%
2.50E+08	4.47E+08	79%	3.24E+08	30%
1.25E+08	2.65E+08	112%	1.63E+08	31%
6.25E+07	1.14E+08	83%	8.59E+07	37%

Table 1: Comparison of calculated concentrations (average of three measurements) and accuracy of unknowns using ELISA and Gator®. The calibration curves were created for each method using AAV2 standards. For ELISA the stock at 1E+11 was diluted to bring it down to the specified dynamic range supported by the kit. Overall demonstrates better accuracy compared to ELISA.

AAV2 KNOWN VS ELISA VS GATOR®

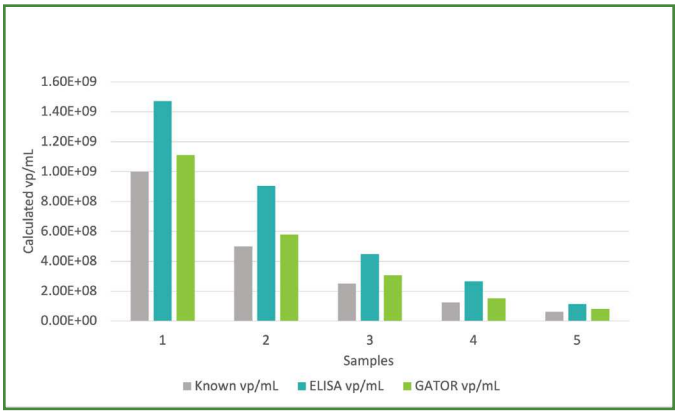

Figure 3: Comparison of ELISA and Gator® calculated concentrations for AAV2 (average of three measurements). Gator® demonstrates better accuracy.

CORRELATION OF ELISA AND GATOR® FOR AAV2

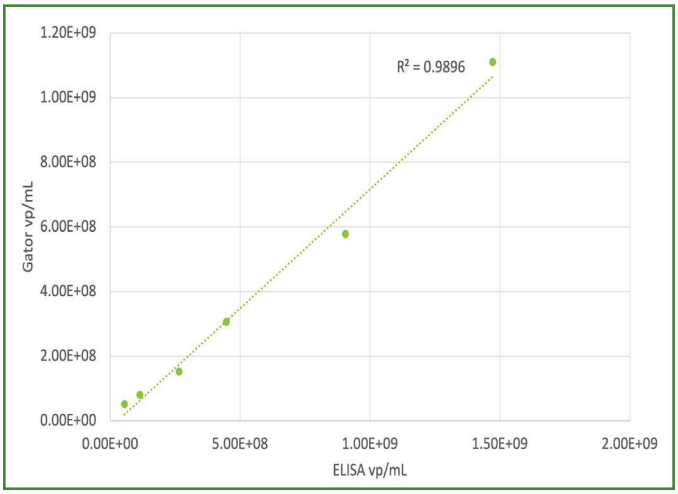

Figure 4: Correlation of ELISA and Gator® calculated concentrations for AAV2. A good correlation between the two methods is observed.

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Comparison of Gator® BLI and Progen ELISA for Quantitation of AAV Serotype 5

% ERROR OF ELISA AND GATOR® FOR AAV5

Known vp/mL	Progen ELISA vp/mL	Progen ELISA % Error	Gator vp/mL	Gator % Error
1.00E+10	1.10E+10	10%	9.57E+09	-4%
5.00E+09	6.72E+09	34%	5.02E+09	0%
2.50E+09	3.94E+09	58%	2.83E+09	13%
1.25E+09	2.31E+09	85%	1.64E+09	31%
6.25E+08	1.16E+09	86%	1.06E+09	70%

Table 2: Comparison of calculated concentrations (average of three measurements) and accuracy of unknowns using ELISA and Gator®. The calibration curves were created for each method using AAV5 standards. For ELISA the stock at 1E+11 was diluted to bring it down to the specified dynamic range supported by the kit. Overall the Gator® demonstrates better accuracy compared to ELISA.

AAV5 KNOWN VS ELISA VS GATOR®

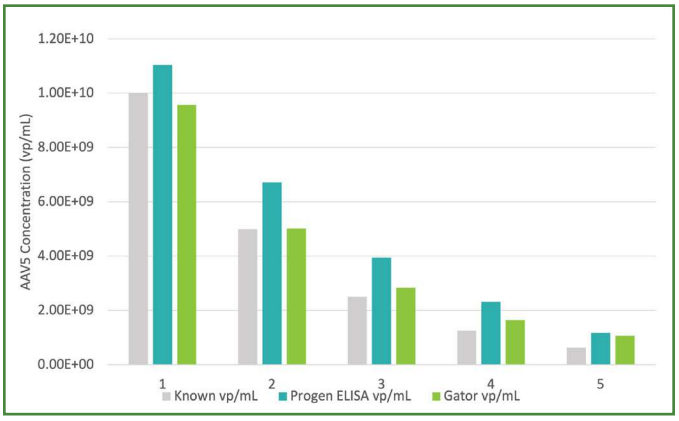

Figure 5: Comparison of ELISA and Gator® calculated concentrations for AAV5 (average of three measurements). Gator® demonstrates better accuracy.

CORRELATION OF ELISA AND GATOR® FOR AAV5

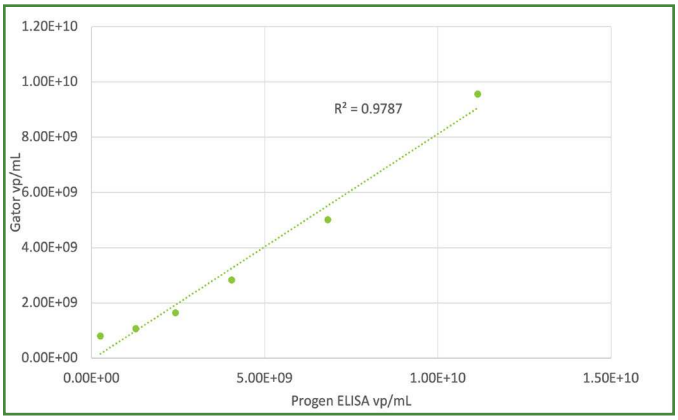

Figure 6: Correlation of ELISA and Gator® calculated concentrations for AAV5. A good correlation between the two methods is observed.

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Comparison of Gator® BLI and Progen ELISA for Quantitation of AAV Serotype 8

% ERROR OF ELISA AND GATOR® FOR AAV8

Known vp/mL	Progen ELISA vp/mL	Progen ELISA % Error	Gator vp/mL	Gator % Error
5.00E+08	4.77E+08	-5%	4.44E+08	-11%
2.50E+08	2.60E+08	4%	2.17E+08	-13%
1.25E+08	1.30E+08	4%	9.43E+07	-25%
6.25E+07	5.84E+07	-7%	5.02E+07	-20%
3.13E+07	2.45E+07	-22%	2.72E+07	-13%
1.56E+07	1.56E+07	0%	1.49E+07	-5%

Table 3: Comparison of calculated concentrations (average of three measurements) and accuracy of unknowns using ELISA and Gator®. The calibration curves were created for each method using AAV8 standards. For ELISA the stock at 2E+11 was diluted to bring it down to the specified dynamic range supported by the kit. Overall the Gator® demonstrates better accuracy compared to ELISA.

AAV8 KNOWN VS ELISA VS GATOR®

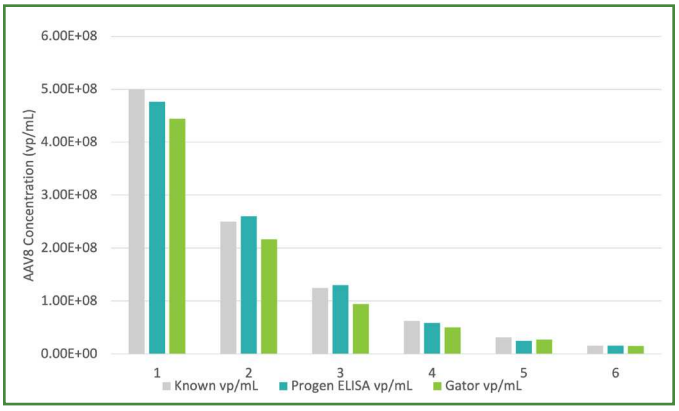

Figure 7: Comparison of ELISA and Gator® calculated concentrations for AAV8 (average of three measurements). Gator demonstrates better accuracy.

CORRELATION OF ELISA AND GATOR® FOR AAV8

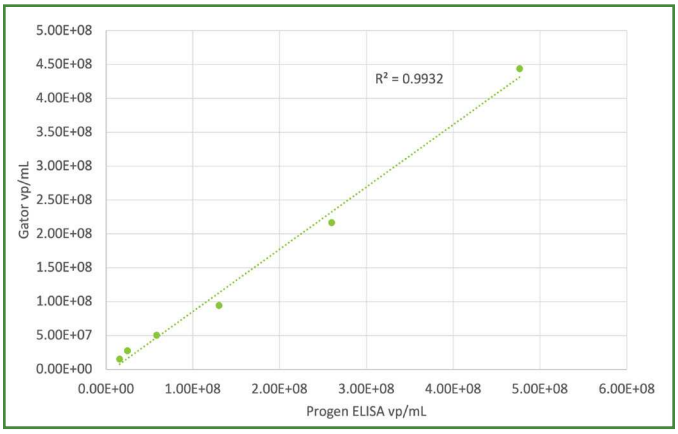

Figure 8: Correlation of ELISA and Gator® calculated concentrations for AAV8. A good correlation between the two methods is observed.

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GATOR® AND ELISA WORKFLOW COMPARISON

Figure 9: Number of steps, hands-on time and total time needed for each step. The Gator® platform demonstrates much shorter run times and hand-on time.

CONCLUSION

- The Gator® solution comprising of Gator® AAVX probes and Gator® Plus system is capable of accurate and reproducible quantitation of AAV serotypes 2, 5, 8 and 9
- The accuracy of Gator® platform is superior to ELISA
- The analysis time of 26 min /96 samples is much shorter than ELISA
- Good correlation with ELISA kit
- Plug and play with little hands-on time
- Eliminates errors associated with dilutions performed for ELISA

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