Comparison of Gator® BLI and Progen ELISA for Quantitation of AAV Serotypes
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INTRODUCTION
Determination of total capsid titer is one of the critical quality attributes for AAVs used in gene therapy.

This poster presents quantitation of AAV serotype 2, 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 iDS, Molecular Devices. The AAV serotype standards were purchased from Virovek, Newark, CA.

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

VERIFICATION OF AAV9 ELISA LINEARITY
Correlation between ELISA and Gator calculated concentrations for AAV9.

CORRELATION OF ELISA AND GATOR® FOR AAV8
Correlation between ELISA and Gator calculated concentrations for AAV8.

CALCULATED CONCENTRATION OF ELISA AND GATOR® FOR AAV8

<table>
<thead>
<tr>
<th>Known vp/mL</th>
<th>Progen ELISA vp/mL</th>
<th>Gator vp/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0E+08</td>
<td>4.77E+08</td>
<td>4.4E+08</td>
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<tr>
<td>2.5E+08</td>
<td>2.60E+08</td>
<td>2.17E+08</td>
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<tr>
<td>1.25E+08</td>
<td>1.30E+08</td>
<td>9.43E+07</td>
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<tr>
<td>6.25E+07</td>
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<tr>
<td>1.56E+07</td>
<td>1.56e+07</td>
<td>1.49E+07</td>
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</tbody>
</table>

COMPARISON WITH OTHER METHODS
Gyrolab: Wide dynamic range but indirect measurement with multiple steps
Octet: Application note shows narrow range and self prepare the probes
ELISA: Highly sensitive, too many steps, multiple kits needed and multiple dilutions
Gator: Wider dynamic range, ready-to-use probe, much less dilution

AAV9 CORRELATION - GATOR® AAVX PROBES VS AAV9 ELISA
Correlation between Gator and ELISA assay for AAV9.

AAV8 KNOWN VS ELISA VS GATOR®
Correlation of ELISA and Gator calculated concentrations for AAV8.

CALCULATED CONCENTRATION OF ELISA AND GATOR® FOR AAV2

<table>
<thead>
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<th>Known vp/mL</th>
<th>Progen ELISA vp/mL</th>
<th>Gator vp/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00E+09</td>
<td>1.47E+09</td>
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<tr>
<td>5.00E+08</td>
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<tr>
<td>6.25E+07</td>
<td>1.14E+08</td>
<td>8.59E+07</td>
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</tbody>
</table>

AAV2 KNOWN VS ELISA VS GATOR®
Correlation of ELISA and Gator calculated concentrations for AAV2.

COMPARISON WITH OTHER METHODS
Gyrolab: Wide dynamic range but indirect measurement with multiple steps
Octet: Application note shows narrow range and self prepare the probes
ELISA: Highly sensitive, too many steps, multiple kits needed and multiple dilutions
Gator: Wider dynamic range, ready-to-use probe, much less dilution

CONCLUSION
• The Gator® solution comprising of Gator® AAVX probes and Gator® Plus system is capable of accurate and reproducible quantitation of AAV serotypes 2, 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