

# Next Gen BLI-based AAV Solutions for Advancing Gene Therapy

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## INTRODUCTION

Gene therapy has the potential to treat a host of diseases from cancer to neurodegenerative disorders. Disease caused by mutated or defective genes can be treated by therapeutic genes that are packaged and delivered to target cells via viral vectors such as the adeno-associated virus (AAV).

As vectors, AAVs are non-integrating and non-immunogenic, which reduces the risk for insertional mutagenesis in the host genome or an immune response. Thus, AAV vectors are one of the most investigated vehicles for gene delivery.

The production of AAV vectors has challenges. The viral particles/capsids are a mix of fully loaded and less ideally, partially loaded and empty capsids.

AAV production requires accurate, precise, and high throughput determination of capsid titer and empty versus full (E/F) content ratio. These features ensure enhanced efficacy, the safety of AAV gene therapy products, and good quality control to promote the desired therapeutic effect.

Gator Bio's Next Gen BLI-based AAV solutions determines AAV capsid titer and empty versus full content without relying on additional techniques (e.g., ELISA, qPCR, ddPCR, AUC, and TEM) to obtain data on different AAV critical quality attributes (CQA).

Here, we present data from our AAV portfolio, including (i) AAVX and AAV9 Quantification, (ii) High Sensitivity (HS) AAV Quantification, (iii) AAV E/F Ratio Determination, and (iv) AAV Neutralizing Antibody Characterization through the use of purified and crude AAV samples.

## FEATURES OF OUR TOTAL AAV SOLUTION

- Automated and robust quantification of serotypes 1-8, 10, and chimeric serotypes in the range of 1E+09 - 1E+14 vp/mL
- Automated and highly specific quantitation of AAV9 in the range of 3E+09 - 1E+14 vp/mL
- Automated high sensitivity AAV quantification for serotypes 1-8 and 10, and chimeric serotypes in range of 5E+06 - 5E+14 vp/mL\*
- High throughput and fully automated empty vs full (E/F) determination in the range of 1E+10 - 1E+14 vp/mL
- Kinetic characterization of AAV neutralizing antibodies

\*Concentrations above 5E+10 vp/mL will have to be diluted to fall within the assay's liner range (5E+06 - 5E+10 vp/mL)

## 1a. AAV QUANTIFICATION USING AAVX PROBES

- AAVX probes bind to AAV serotypes 1-8 and 10, and novel serotypes.
- AAV9 probes use AAV9 specific nanobody for selective capture of AAV9.
- Quantitation is based on the binding rate of the AAV capsid of interest to the probe surface.
- Compatible with crude samples such as cell culture media and lysates, eliminating the need for AAV purification prior to assay.
- Probes are reusable at least 10 times with regeneration and without a loss in performance, significantly reducing costs.

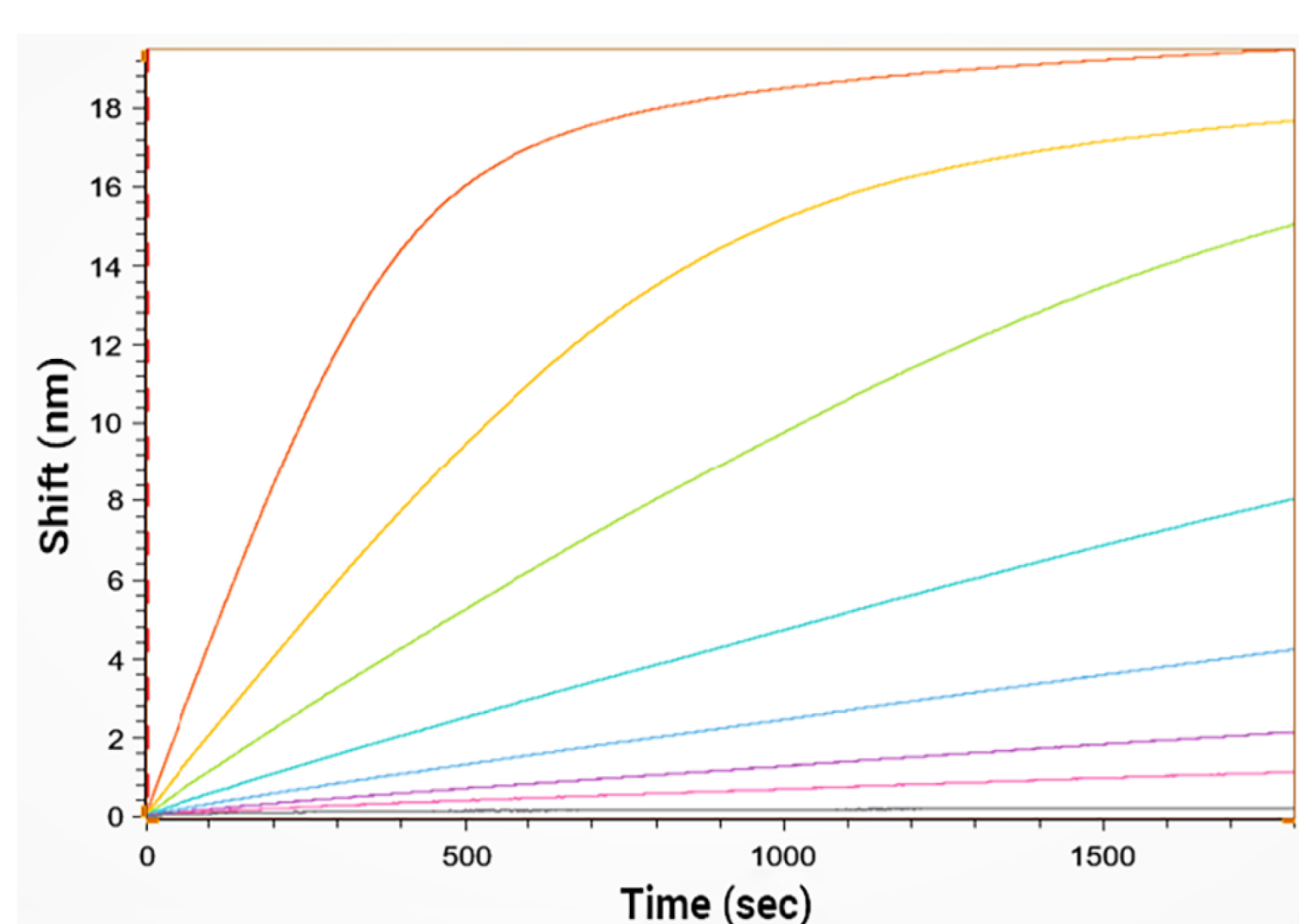


Figure 1. Capture of AAV2 serotype using AAVX probes. The concentration range is 1.21E+09 - 3.33E+13 vp/mL with a 1:3 dilution series in Quantitation (Q) Buffer.

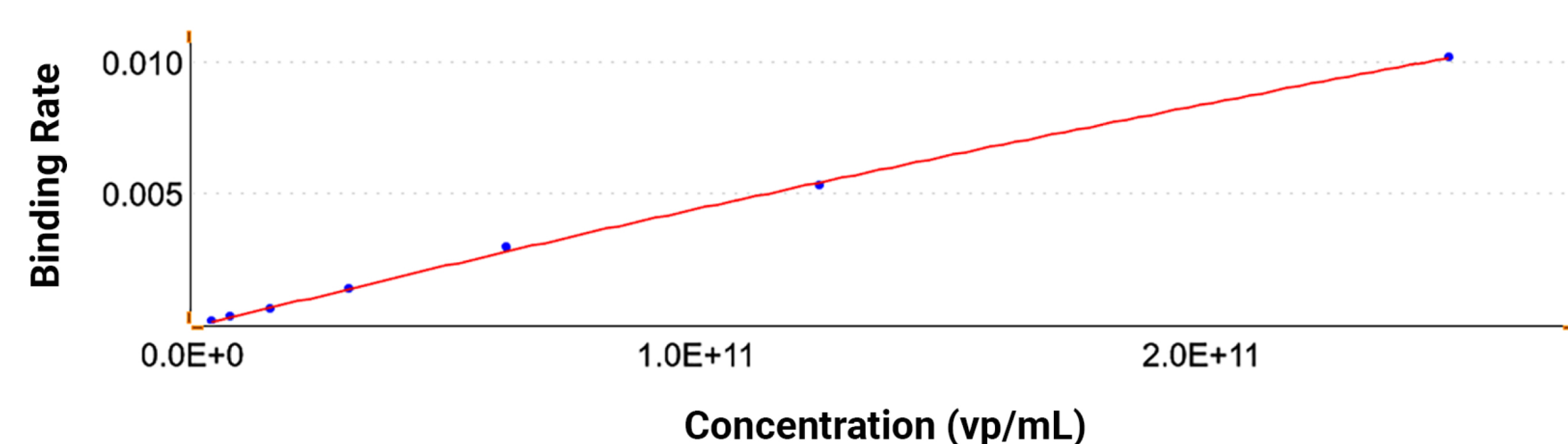


Figure 2. Standard curve for AAV2 is generated by using the GatorOne analysis software.

## 1b. CAPTURE OF AAV1-8 USING AAVX PROBES

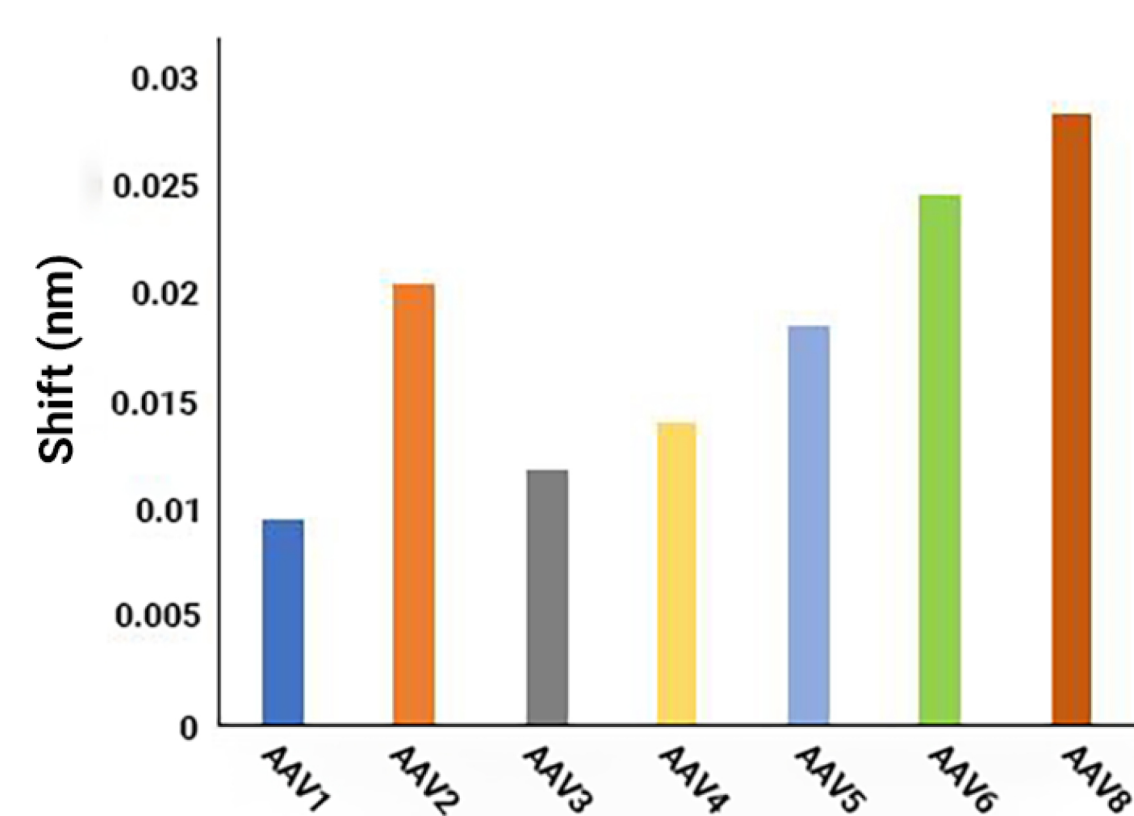


Figure 3. The binding of AAV serotypes 1-8 using AAVX probes at a concentration of 2E+11 vp/mL. Serotypes are purchased from www.virovek.com.

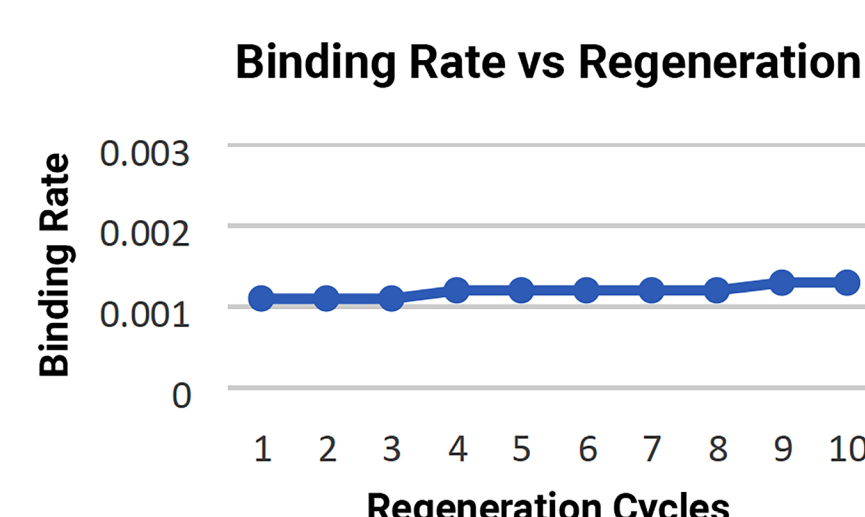


Figure 4. There is no loss in performance after 10 regenerations using the same AAVX probe.

## 1c. QUANTIFICATION OF SEROTYPE 9 USING AAV9 PROBES

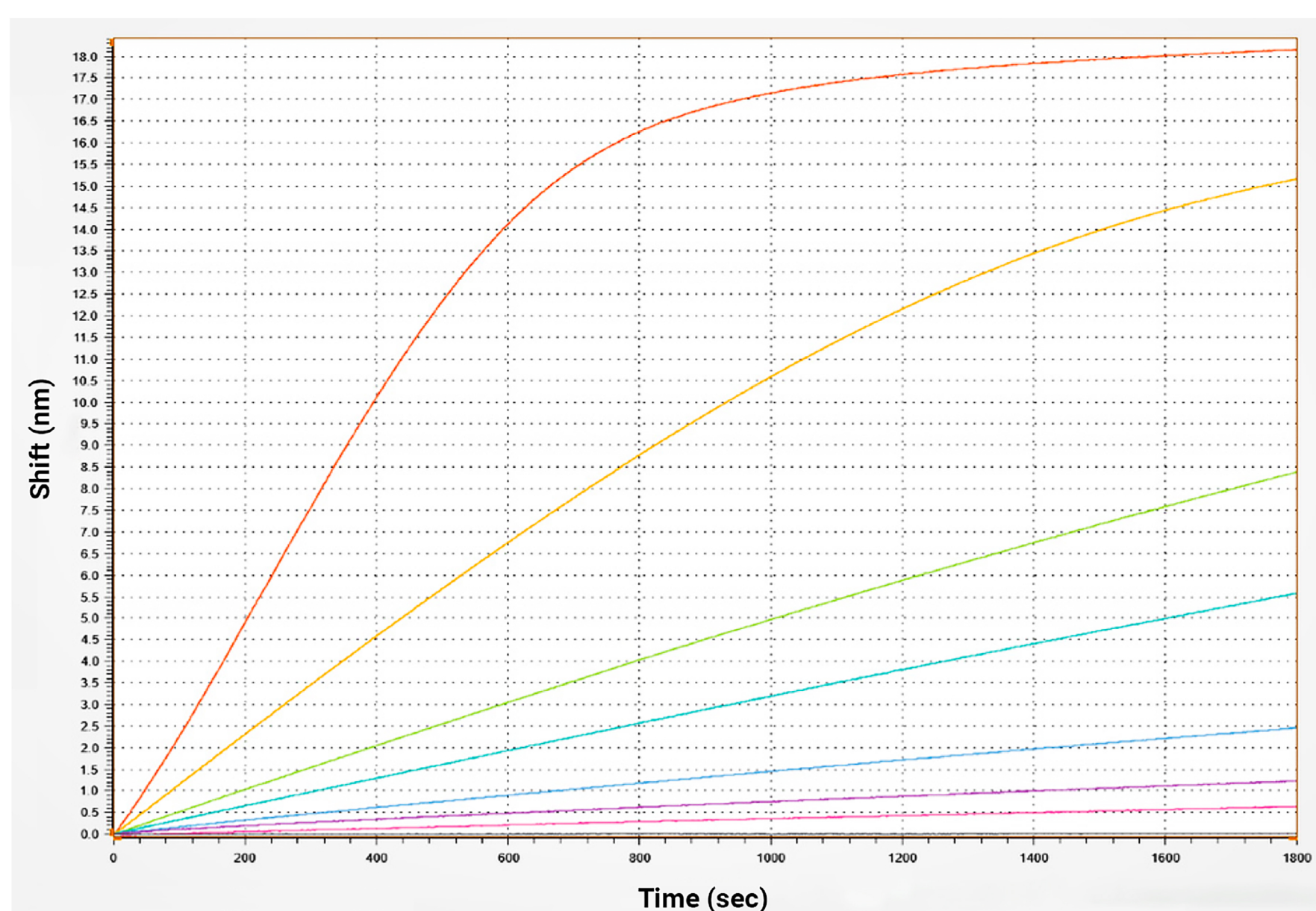


Figure 5. Capture of serotype 9 using AAV9 specific probes. The concentration range is 2.5E+11 - 3.5E+09 vp/mL with a 1:2 dilution series in Q Buffer.

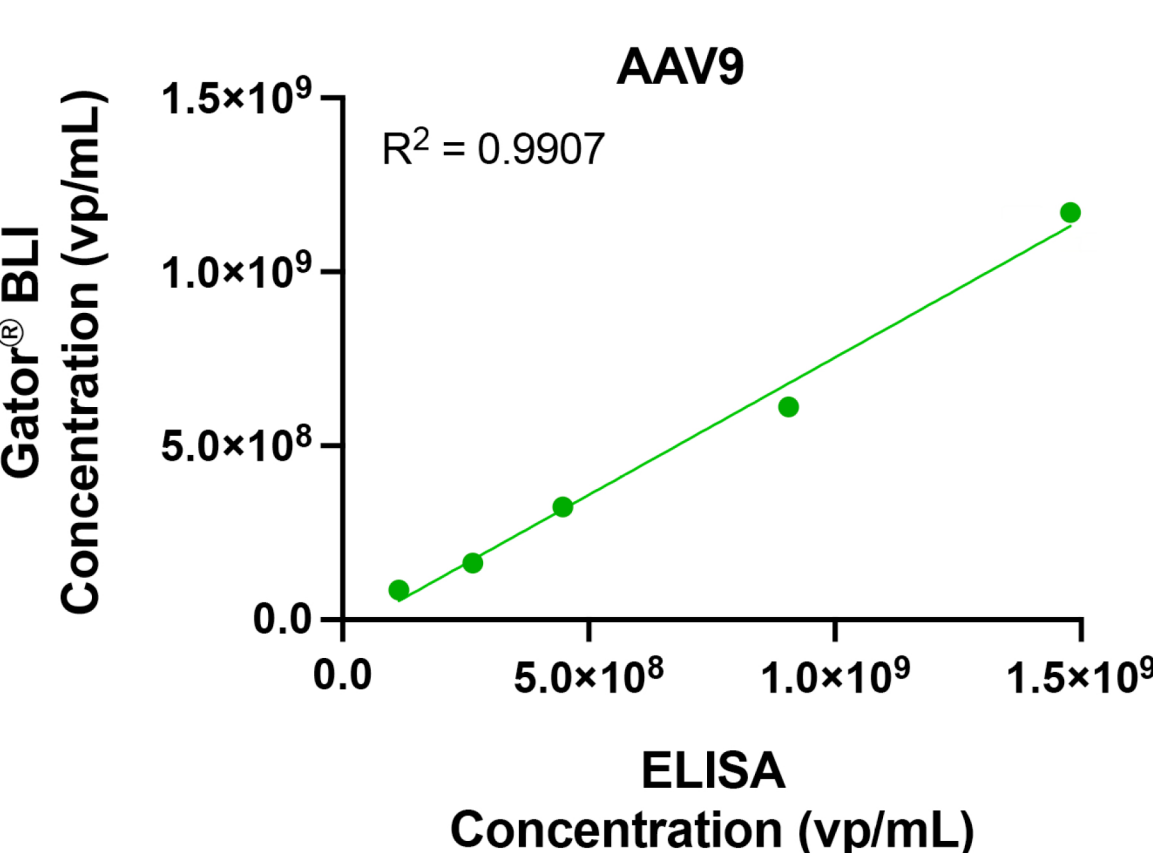


Figure 6. Correlation between the quantification of AAV9 obtained by BLI-based AAVX probes versus ELISA.

## 2. HIGH SENSITIVITY AAV PROBES

- Enables increased sensitivity up to 5E+06 vp/mL, while still offering Gator Bio's automation, affordability, decreased hands-on time, low sample volume, and high precision.
- High sensitivity quantification of AAV serotypes 1-8, 10, and chimeric serotypes.
- Enhanced sensitivity is powered by patented signal amplification technology involving horseradish peroxidase (HRP).

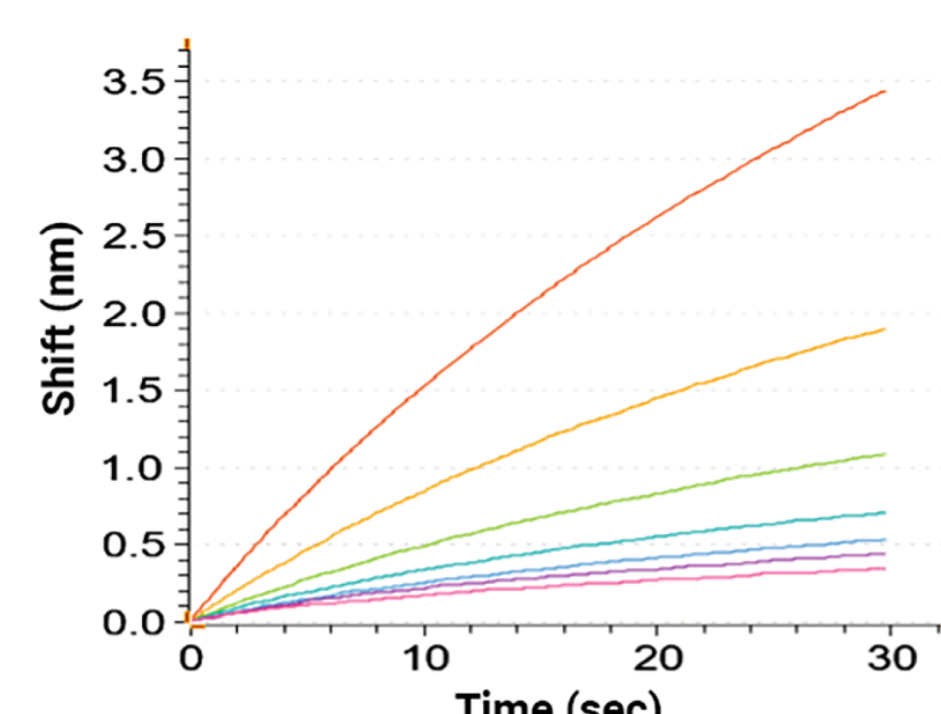


Figure 7. Sensogram showing nm shift for AAV5 ranges from 3.13E+06 - 1E+08 vp/mL with a 1:2 dilution

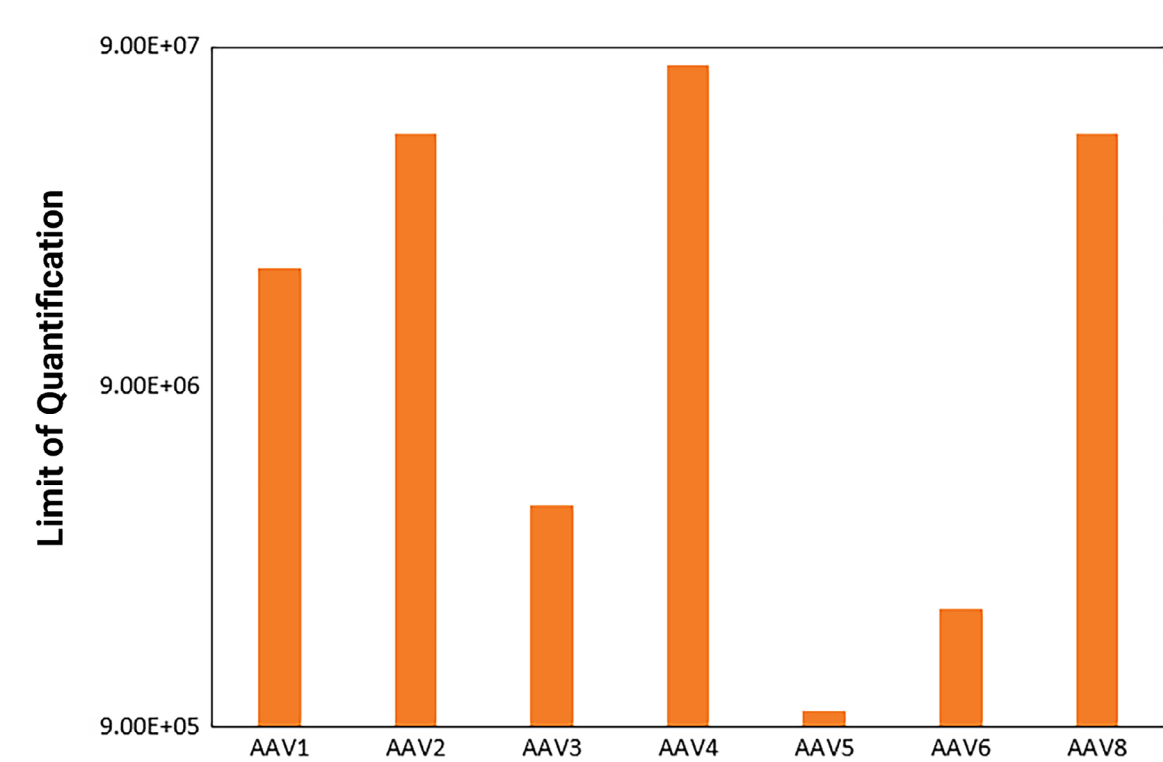


Figure 8. The Limit of Quantification (vp/mL) for AAV1-8 serotypes. Serotypes are purchased from www.virovek.com.

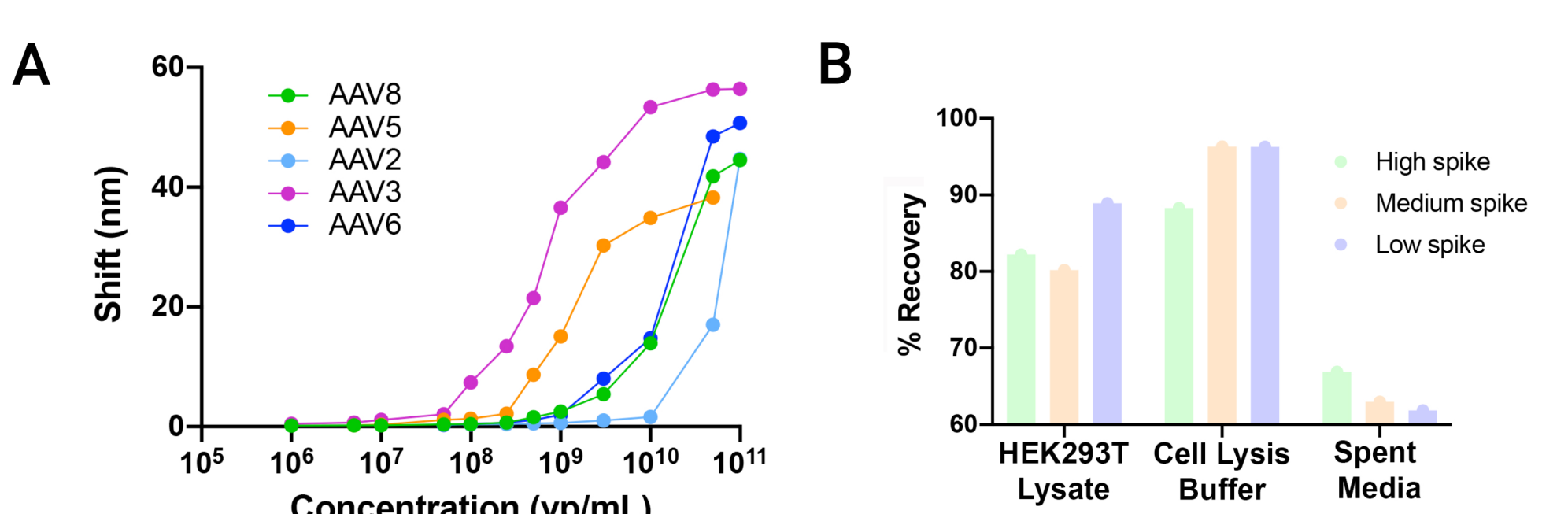


Figure 9. (A) HS AAV dynamic range for AAV serotypes. (B) Recovery of AAV spiked at high (1E+08 vp/mL), medium (5.33E+07 vp/mL), and low (1E+07 vp/mL) concentrations in various sample matrices.

## 3. E/F AAV RATIO DETERMINATION

- The E/F Ratio Determination Kit detects total single-stranded (ss) DNA content in AAV capsids with three steps: (i) AAV capture using AAVX probes, (ii) lysis for releasing the ssDNA, and (iii) capture of ssDNA on ssDNA probes.
- The kit offers the ease of using a single assay for E/F ratio determination without the use of other methods, and providing automation, speed, accuracy, and precision.

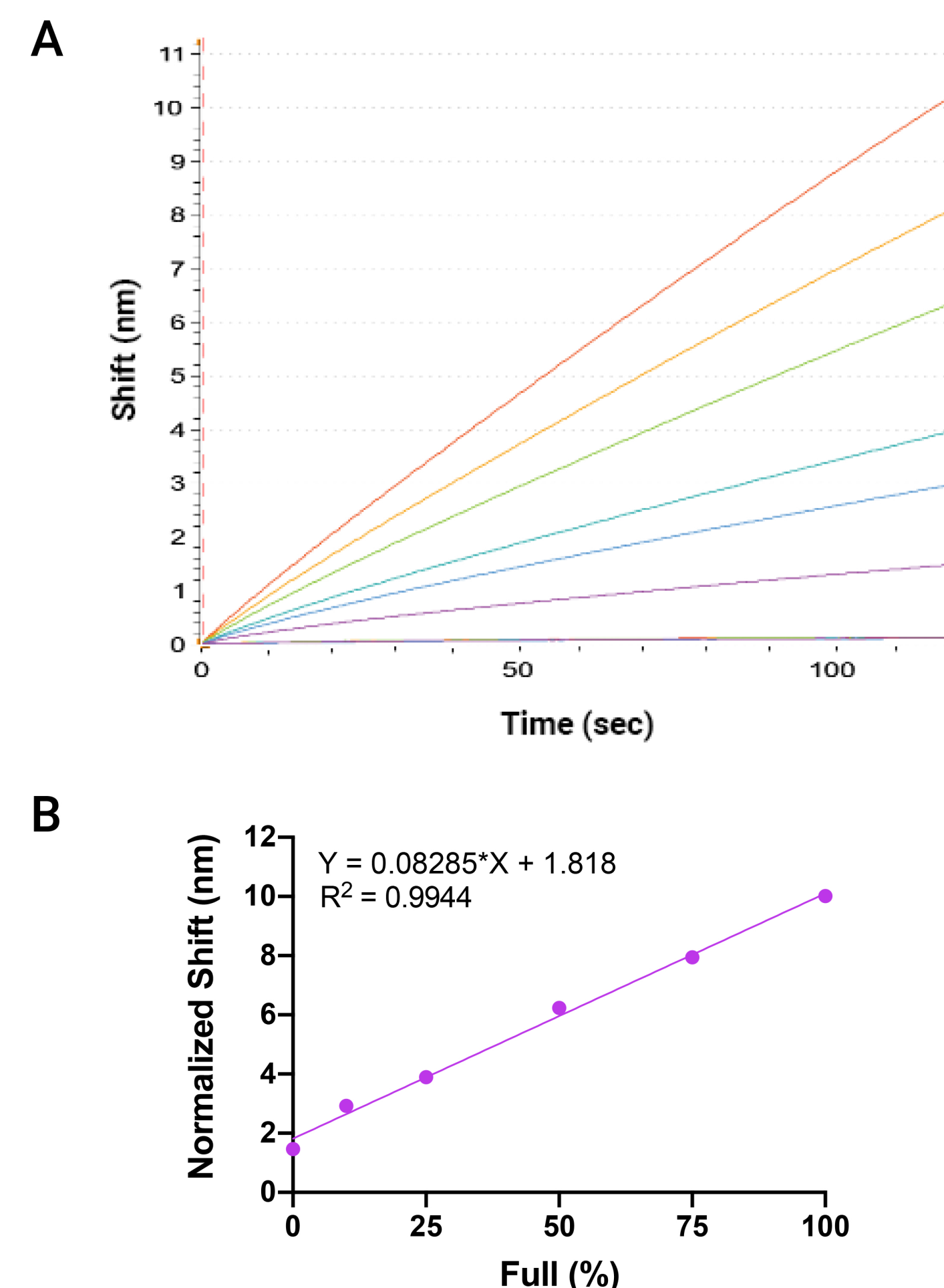


Figure 10. (A) Sensogram showing the capture of ssDNA using ssDNA probes. (B) Standard curve for percent (%) full of AAV8 capsids.

## 4. AAV NEUTRALIZING ANTIBODY CHARACTERIZATION WITH AAV BIOSENSORS

- AAVX and AAV9 probes allow for characterization of neutralizing antibodies through kinetic analysis of antibody interactions with AAV particles captured on AAVX/AAV9 probes.
- GatorOne analysis software generates  $K_D$  values, and various antibodies can be ranked based on their affinity to the AAV particles.

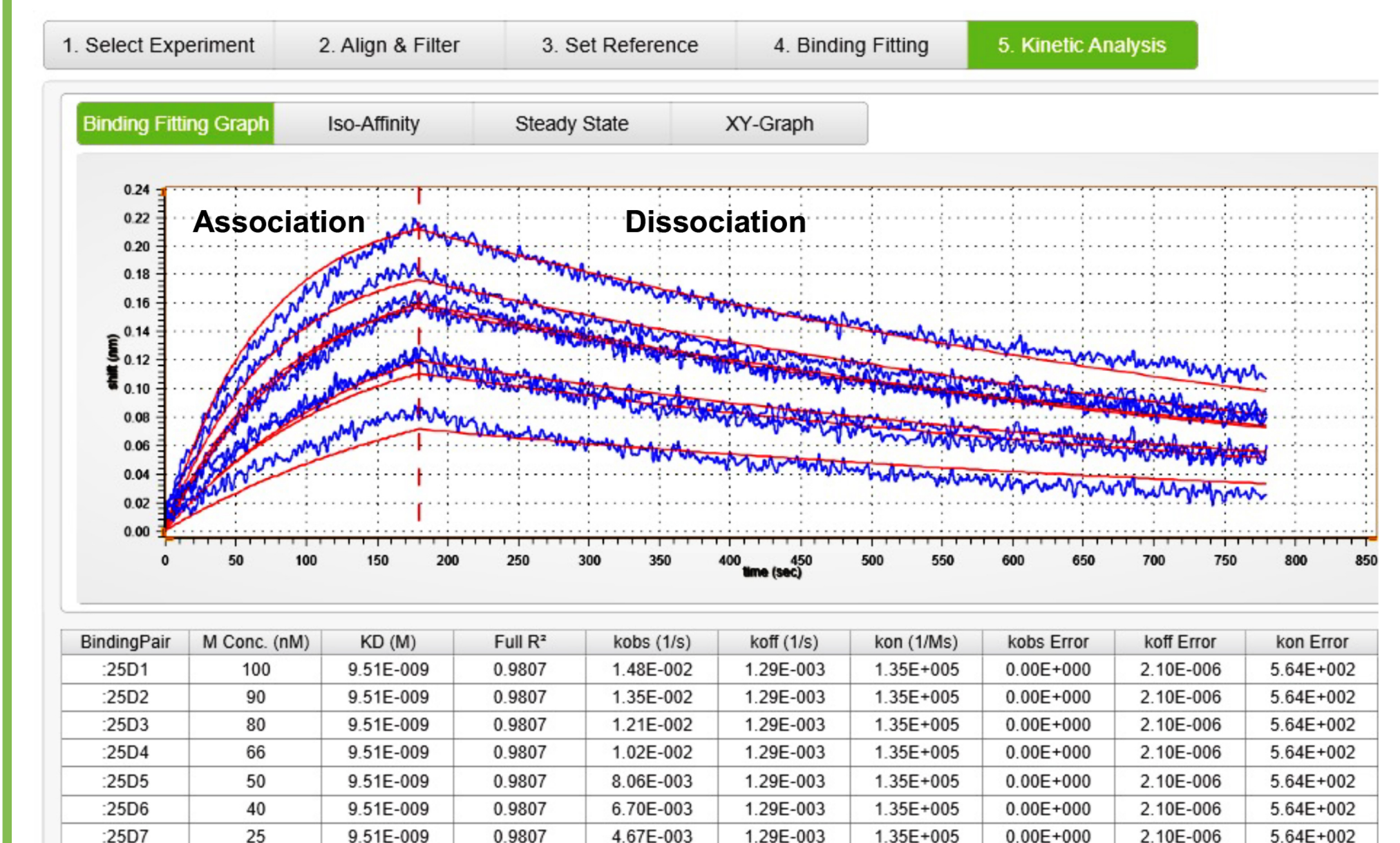
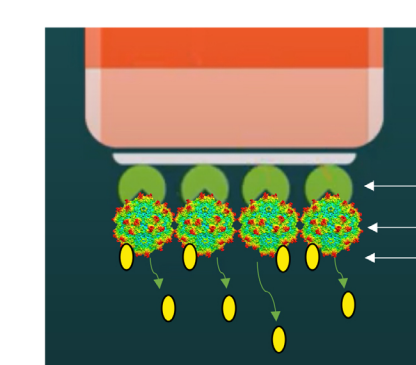


Figure 11. Neutralizing antibody Fab fragments dissociating from AAV particles captured onto AAVX probes. Dissociation curves and  $K_D$  values are generated by the GatorOne analysis software.

## GATOR® GENE THERAPY SOLUTION GUIDE

Gator® Biosensor / Kit	Function	Applications	Dynamic Range	Regeneration
AAVX	Binds serotypes AAV1-10 and novel serotypes	Q	10 <sup>9</sup> - 10 <sup>14</sup> vp/mL	Yes
AAV9	Binds specifically to the AAV9 serotype	Q	10 <sup>9</sup> - 10 <sup>14</sup> vp/mL	Yes
High Sensitivity AAV Kit	High sensitivity quantification of AAV serotypes 1-8, 10, and chimeric serotypes	Q	5 X 10 <sup>6</sup> - 1 X 10 <sup>14</sup> vp/mL*	No
High Sensitivity AAV9 Kit	High sensitivity quantification of AAV serotype 9	Q	5 X 10 <sup>6</sup> - 1 X 10 <sup>14</sup> vp/mL*	No
Empty / Full Ratio Determination Kit	% Full determination	Ratio determination	10 <sup>10</sup> - 10 <sup>14</sup> vp/mL	No

\*Concentrations above 5E+10 vp/mL will need to be diluted in order to fall within the assay's liner range (5E+06 - 5E+10 vp/mL).

## SUMMARY

- Gator® AAV probes and kits provide a total solution for gene therapy.
- Features of the probes and kits include:
  - A wide dynamic range of 5E+06 - 1E+14 vp/mL
  - A single assay for E/F content ratio determination
  - Matrix compatibility that is ideal for upstream analysis
  - Affordability from a single technology for AAV quantitation and content ratio determination
- Gator® BLI-based solutions for AAV capsid titer and content are advantageous over current methods due to automation, high dynamic range, precision, and easy integration into upstream use.