PRODUCT INSERT

Gator™ AAVX Probe

Catalog No.160017

Overview

Gator™ AAVX probes are useful for measuring the concentration of different serotypes of AAV that includes AAV1 to AAV8, and AAVrh10 as well as synthetic serotypes. This product was developed with the CaptureSelect™ (Thermo Fisher Scientific) high affinity and high specificity anti-AAVX antibody. In conjunction with the Gator Prime and Gator Plus systems, AAVX probe provides a rapid and label-free method for the quantitation of AAV serotypes. The high specificity of the antibody-based biosensor enables the direct capture and quantitation of different serotypes of AAV in crude lysates, column eluates, cell lysates and cell culture supernatants, serving as an alternative to traditional time-consuming analytical methods, such as HPLC, ELISA, dPCR, etc.

Materials required

AAVX Probe	Catalog No.160017
Max Plate	Catalog No. 130062
Black Plate	Greiner 655209 (96 well) Greiner 781209(384 well)
Quantitation (Q) Buffer	Catalog No. 120010
Regeneration Buffer (No Salt)	Catalog No. 120008

Storage

Store AAVX probes (Catalog number:160017) at room temperature in the foil pouch, ensuring zipper is fully sealed to avoid humidity/moisture contamination. In high-humidity environments, storage inside a dry cabinet is recommended.

Application Summary

- Dynamic range: 1 x 10^9–1 x 10^{13} vp/mL for most AAV serotypes.
- Throughput: 8 samples in ~10 minutes, 96 samples in ~120 minutes
- Limit of detection: typically 5 x 10^8 particles/mL (serotype dependent)

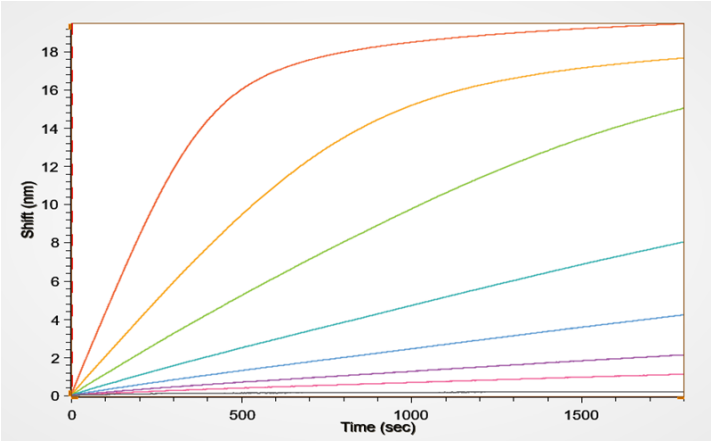

Figure 1: Capture of AAV2 serotype on the anti-AAVX probes. The concentration range is 1.21x 10^9 vp/mL – 3.33 x 10^{13} vp/mL with 1:3 dilution series in Q buffer (assay protocol: 10 min at 1000 rpm).

AAVX quantitation assay principle

- The AAVX assay for determining total virus capsid concentration is based on the rate of binding of AAV of interest to the biosensor surface.
- Different AAV concentrations result in different binding rates.
- Gator software calculates the binding rates from standards with known values to generate a standard curve — the binding rate of each standard is proportional to its concentration.
- Concentrations of experimental samples are calculated based on their binding rate compared to that of the known concentrations that make up the standard curve

General Methods

Sample Volume

- Black Plate (96 well plate): 200 µL (180 µL minimum)
- Black Plate (384 well plate): 100 µL (80 µL minimum)
- Max Plate: 250 µL (280 µL maximum)

Pre-wet Conditions

- 250 µL of buffer diluent in Max Plate, 10 min at 1000 rpm

Tips for Optimal Performance

For the best performance, it is recommended to regenerate the probes using Regeneration Buffer - No Salt (Cat No. 120008) prior to use.

Gator Bio, Inc. 2454 Embarcadero Way, Palo Alto, CA 94303, USA · +1 855 208 0743 · info@gatorbio.com · **GatorBio.com**

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Regeneration

The underlying surface chemistry of the AAVX probe is robust and stable over a broad range of pH. Most AAV interactions binding to the AAVX probes can be disrupted using low pH buffers (pH 1–3). The ideal condition for regenerating the AAVX probe is in 10 mM Glycine pH 1.7 with 5 sec at 1000 rpm. The probes can be reused at least 10 times without showing significant loss of binding to AAV. AAV serotypes and sample matrix affect regeneration performance, therefore protocol development and formulation studies are recommended for optimal regeneration conditions.

Tips for quantitation assays

• Pre-wet the AAVX probe for at least 10 minutes in buffer/matrix that is an exact match to buffer/matrix of the samples being analyzed. This will minimize background response from non-specific binding to the biosensor.

• When generating standard curves or running unknowns, always run a blank, which should be used as a reference for background subtraction during the data analysis.

• For accurate results, a standard curve must be generated using the same AAV serotype as the sample(s) to be quantitated.

• The standards should be diluted in a buffer matrix that is an exact match of the unknown sample(s).

• User can save standard curve in the software and re-loaded it for subsequent experiments.

• The concentration of sample(s) being analyzed should fall within the concentration range of the standard curve for accurate quantitation.

Titer Level	Known Conc. (vp/mL)	Average Binding Rate	Average Calculated concentration (vp/mL)	% Recovery	% CV (n = 3)
High	2.00×10^{12}	0.11069	2.03×10^{12}	102	1.50
Medium	5.10×10^{10}	0.0045	5.27×10^{10}	103	1.81
Low	8.30×10^{8}	0.00010	8.30×10^{8}	100	10.9

Table 1: Average calculated concentration and precision of AAV5 samples.

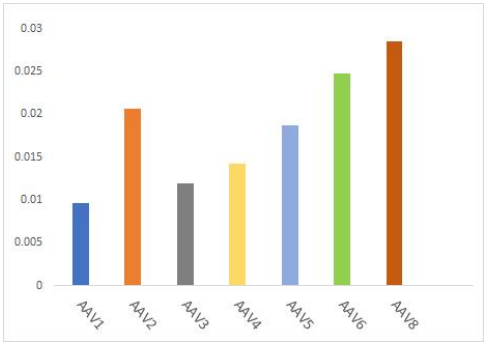

Figure 2: Different AAV serotypes binding to the AAVX probes are compared at 2×10^{11} vp/mL. All serotypes were purchased from www.virovek.com

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