PRODUCT INSERT

Gator™ AAV9 Probes

Catalog No. 160021

OVERVIEW

Gator™ AAV9 ready-to-use probes are manufactured with a high affinity and high specificity anti-AAV9 nanobody which is pre-immobilized on a Gator™ probe. In conjunction with the GatorPrime™ and GatorPlus™ instruments, the AAV9 probe provides a rapid and label-free method for quantitation of AAV9 serotype. The high specificity of the nanobody-based biosensor enables the direct capture and quantitation of different AAV9 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. In this quantitation technique, the total AAV9 capsid is determined.

MATERIALS REQUIRED

AAV9	Catalog No.160021
Max Plate	Catalog No. 130062
Black Plate	Greiner 655209 (96 well)
	Greiner 781209 (384 well)
Regen Buffer (No Salt)	Catalog No. 120008

STORAGE

Store AAV9 probes 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: 3 x 10^9–1 x 10^{13} vp/mL for AAV9
- Throughput: 8 samples in 4 min, 96 samples in 26 min
- LoD: 1 x 10^9 vp/mL
- Crude sample tolerant
- Stable over broad pH range
- Cost effective - Reusable at least 10 times

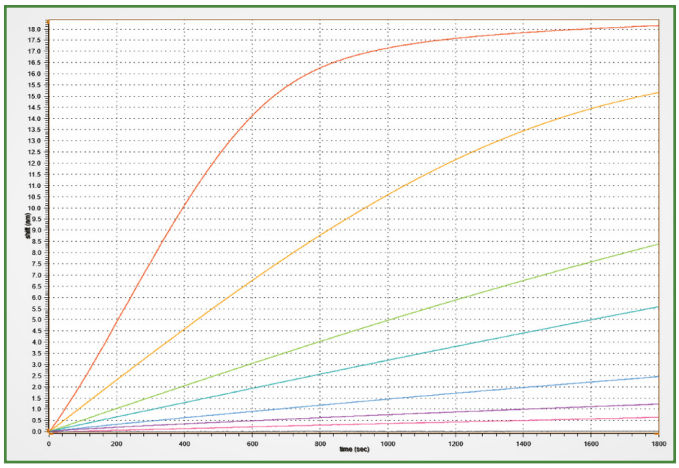

Figure 1: Capture of AAV9 on the AAV9 probes. The concentration range is 2.5 x 10^{11} – 3.5 x 10^9 vp/mL in 2 fold dilution series in quantitation buffer, (30 min/1000 rpm).

GENERAL METHODS

Sample Volume

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

Pre-wet Conditions

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

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AAV9 QUANTITATION ASSAY PRINCIPLE

- The AAV9 assay for determining total virus capsid concentration is based on the rate of binding of AAV9 to the biosensor surface. The GatorOne™ Software calculates the binding rates from standards with known values to generate a standard curve — the binding rates 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.

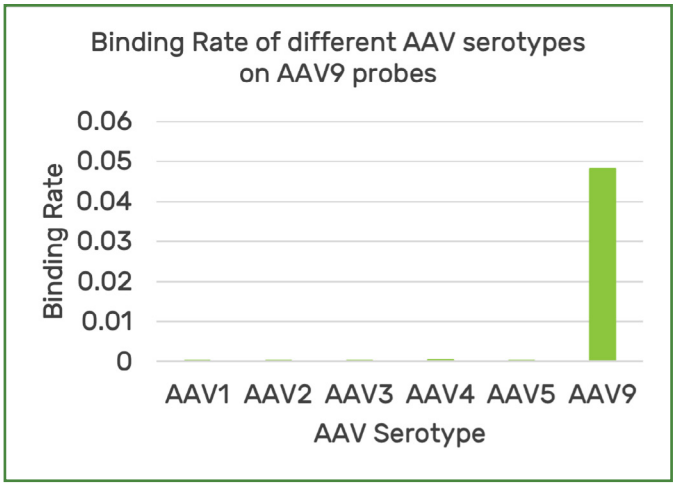

Figure 2: Different AAV serotypes cross-reactivity to the AAV9 probes are compared at 2 x 10^{11} vp/mL in buffer diluent.

REGENERATION

The underlying surface chemistry of the AAV9 probe is robust and stable over a broad range of pH. Binding to the AAV9 probes can be disrupted using low pH buffers (pH 1–3). The ideal condition for regenerating the AAV9 probe is 10 mM Glycine pH 2.0 with 5 sec and 1000 rpm. The probes can be reused in buffer diluent for at least 10 times without showing significant loss of binding to AAV9. The buffer diluent, media etc plays an important role, hence it is best to optimize regeneration condition for each assay to achieve the desired outcome.

Titer Level	Known Conc. (vp/mL)	Average Calculated Conc. (vp/mL)	% CV (n = 4)
High	1.00 x 10^{12}	1.04 x 10^{12}	3.7
Medium	5.00 x 10^{10}	4.66 x 10^{10}	5.1
Low	2.50 x 10^{9}	2.49 x 10^{9}	4.5

Table 1: Average calculated concentration and % CV (N=4) of AAV9 serotype.

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TIPS FOR QUANTITATION ASSAYS

- Pre-hydrate AAV9 probe for at least 10 minutes in assay buffer or matrix that exactly matches the sample to be analyzed. This will minimize background response from non-specific binding to the biosensor
- Always run blank control when generating standard curves or running unknowns, which should be used as a reference for background subtraction during the data analysis
- For accurate results, a standard curve must be generated using AAV9 serotype
- The standards should be diluted in a buffer matrix that exactly matches that of the unknown sample(s)
- Standard curves can be saved on the GatorOne™ Software and reloaded for subsequent experiments, as the standard curve and sample data are obtained with biosensors from same manufacturing lot
- The concentration of sample(s) being analyzed should fall within the concentration range of the standard curve for accurate quantitation

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