

Gator® High Sensitivity (HS) AAV Kit

Part No. 350003

Overview

Gator® High Sensitivity (HS) AAV Kit is a high sensitivity assay to quantify adeno-associated virus (AAV) capsid concentrations for AAV serotypes 1-8 at all stages of viral vector development in mutliple matrices. It combines high sensitivity from a signal amplification step, and ease of use from the inherent simplicity of the Gator® Biolayer Interferometry (BLI) platform. The HS AAV Kit delivers accurate quantitation over a broad dynamic range of 5E+06 to 5E+10 viral particles (vp)/mL for most serotypes. The proprietary surface chemistry makes the quantification of AAV serotypes highly robust and precise.

Performance Summary

- Dynamic range: 5E+06 5E+10 vp/mL
- Limit of Detection (LoD): 1E+06 vp/mL
- Throughput: 8 samples in 35 minutes;
 24 samples in 99 minutes
- Precision: CV <10%

Product Components

HS AAV Detection (Solution A) at 10X	2 mL
HS AAV Amplification (Solution B) at 10X	2 mL
HS AAV Substrate (Solution C) at 1X	25 mL
Q Buffer	350 mL
HS AAV Probes	96 probes

Materials Required

Gator® Max Plate	Part No. 130062	
Gator® BLI 96-Flat Plate	Part No. 130260	
Precision Pipettes	User Supplied	
Sterile Pipette Tips	User Supplied	

Storage

Store HS AAV probes (Part No. 350003) at room temperature (RT) in their foil packaging pouch, ensuring that the zipper is fully sealed to avoid humidity/moisture contamination. Probes are stable at RT for 1 year. Store HS AAV Reagent Set at 4°C; unopened reagents are stable up to 6 months. Once reagents are diluted, the kit is stable at 4°C for 1 month.

General Methods

Sample Volume

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

Prewet Conditions

180 sec at 1000 rpm in Q Buffer

Reagent Preparation

Dilute Solution A and Solution B, each in 18 mL of Q buffer, and mix thoroughly. Diluted solutions are stable for 1 month at 4°C.

Quantification

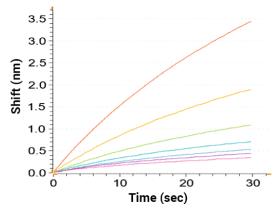


Figure 1. The concentrations of AAV5 in Q Buffer range from 3.13E+06 – 1E+08, serially diluted at 1:2.





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Sample and Standard Preparation

- · Prepare AAV standards in Q Buffer.
- Dilute crude samples in Q Buffer. A 1:10 starting dilution is recommended.
- Use the same sample maxtrix that the samples are in as a Reference, at the same dilution factor in Q Buffer that the rest of the samples are diluted at. Subtract the Reference from the sample readings.
- For a detailed protocol on sample preparation and setting up the HS AAV assay, refer to the Gator® HS AAV Protocol document.
- Test several dilutions of the sample to ensure that the AAV concentrations fall within the recommended dynamic range, as provided in the HS AAV Protocol.

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AAV Kit

Recovery in Crude Samples

AAV5 Conc (vp/mL)	Signal (nm shift)	Calculated Conc (vp/mL)	% CV (Calculated Conc) n=3	% Recovery
1.06E+08	1.93	8.73E+07	2.8301	82.22
5.33E+07	1.04	4.27E+07	2.1964	80.18
1.05E+07	0.38	9.38E+06	7.6104	88.92

Table 3. Signal (nm shift), % CV and % recovery for AAV5 in HEK 293-T cell lysate diluted 1:10 in Q buffer. The recovery for samples spiked with different AAV concentrations is over 80%.