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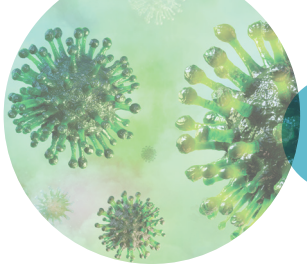

Rapid, at-line AAV virus titer assay

Rapid and accurate methods for the quantitation of Adeno-associated virus (AAV) particles are an unmet need for advancing bioprocessing in gene therapy. Viral capsid titer is commonly measured by ELISA, empty vs. full capsid titer differentiation and ratio is obtained by Analytical Ultracentrifugation (AUC), and viral genome titer is measured increasingly by ddPCR[1]. These methods are generally time consuming and labor-intensive, and are hence not practical for at-line, rapid measurement of viral titer during bioprocessing and manufacturing.

ForteBio has developed a quick, high-throughput and robust AAV2 capsid quantification method, capable of virus titer determination in samples along the purification process. The method accelerates assay of multiple 96-well or 384-well plates in less than 1 hour, saving significant time and labor compared to ELISA and ddPCR assays which require approximately 5 and 8 hours respectively, and involve significant hands-on time at the bench.

Octet® instruments utilize the Bio-Layer Interferometry (BLI) technology in combination with 96- or 384-well sample plates and off-the-shelf biosensor probes to provide rapid and accurate analysis of biomolecular interactions in real time. The Octet platform has been used for both titer and kinetic properties determination of molecules ranging from recombinant proteins to monoclonal antibodies. In virus vaccine studies, Octet systems have been deployed for research on a diverse range of virus and virus-like particles including HIV, influenza virus and Ebola virus[2].

The Octet AAV2 titer assay demonstrated excellent precision and reliability, with very minimal matrix effects relative to ELISA and ddPCR. The method can be extended as a generic viral titer assay for at-line testing of any AAV serotype in a bioprocess setting. The development of the generic method can be achieved by coating the appropriate specific capture ligand on a commercially available biosensor. We postulate that the Octet platform offers near real-time feedback on the bioprocess, saving significant time and resources, thereby enhancing efficiency and productivity for virus manufacturing. Some of the key highlights of the Octet AAV2 quantitation assay are listed in Table 1.

	BLI (Octet RED96e System) + AAV biosensor*	BLI (Octet RED384 System) + AAV biosensor*	BLI (Octet HTX System) + AAV biosensor*	ELISA
Time to results	<1 hr per 96-well plate	<30 min per 96-well plate	5 min per 96-well plate	4–6 hrs per 96-well plate
Operator time	Approx. 10 min	Approx. 10 min robotics compatible	Approx. 10 min Robotics compatible	2–3 hrs
Precision (% CV)	<10%	<10%	<10%	15–20%
Dynamic range	10^8–10^{10} gc/mL	10^8–10^{10} gc/mL	10^8–10^{10} gc/mL	$5x10^7$–$1x10^9$ vp/mL requires series dilutions
Sample type	Crude cell culture supernatant (without centrifugation or filtration)	Crude cell culture supernatant (without centrifugation or filtration)	Crude cell culture supernatant (without centrifugation or filtration)	Cell culture supernatant
Sample preparation	None	None	None	Sample dilution 1:5,000–50,000

Table 1: Comparison of method attributes for AAV2 assay – Octet systems vs. ELISA. The unit gc/mL stands for genome copy per mL. Results from the Octet assay were transferred to gc/mL units on the basis of comparison studies conducted on the same samples by ddPCR.

References

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2 Rejane Petersen, Strategies Using Bio-Layer Interferometry Biosensor Technology for Vaccine Research and Development, *Biosensors (Basel),* 201,7 Oct 31, 7(4), pii: E49, doi: 10.3390/bios7040049.

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