

Application of Size Exclusion Chromatography with Multi-Angle Light Scattering in the Analytical Development of a Preclinical Stage Gene Therapy Program

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Sample

Sample	AAV9
MW	~5Kda
pI	6.5-7.5

Experimental Conditions

Column	SRT SEC-1000, 5 μm , 1000 \AA , 4.6 \times 300 mm
Part Number	215950-4630
Mobile Phase	An isocratic mobile phase of 1XPBS (from 10X PBS) containing 0.01% (vol/vol) pluronic F-68 was used after passing through a 0.2 μm filter.
Flow Rate	0.3 mL/min
Injection	45 μL
Temperature	4 $^{\circ}\text{C}$ sample, 25 $^{\circ}\text{C}$ column
Instrument	HPLC (260/280nm); MALS

500 \AA and 1000 \AA SEC-MALS outperformed the other methods and show good correlation with SV-AUC values of full-to-empty particles, and ddPCR and ELISA measurements for Vector Genomes (VG)/mL and Capsid Particles (CP)/mL, respectively.

Spike Concentration (VG/mL)	Mean Measured VG/mL	%CV	Mean Percent Recovery	Mean Vector Molar Mass (g/mol)	%CV	Mean DNA Molar Mass (g/mol)	%CV	Mean VG/CP Ratio
1.50E+11	1.36E+11	7%	91%	5.24E+06	5%	1.28E+06	5%	0.950
2.50E+11	2.29E+11	3%	92%	5.13E+06	1%	1.23E+06	2%	0.916
5.00E+11	4.91E+11	8%	98%	5.16E+06	4%	1.25E+06	4%	0.926
1.00E+12	9.28E+11	3%	93%	5.02E+06	1%	1.22E+06	1%	0.927
2.50E+12	2.32E+12	5%	93%	5.03E+06	1%	1.22E+06	1%	0.922
1.00E+13	1.04E+13	4%	104%	5.00E+06	0%	1.21E+06	0%	0.918
2.50E+13	2.63E+13	3%	105%	4.98E+06	0%	1.20E+06	0%	0.916

Table 1 – Accuracy By Recovery Data by SEC-MALS